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RAPID COMMUNICATION / COMMUNICATION RAPIDE

Should depensation be dismissed as a possible explanation for the lack of recovery of the northern cod (*Gadus morhua*) stock?

Peter A. Shelton and Brian P. Healey

Abstract: After 7 years of a moratorium on northern cod (*Gadus morhua*), there is no convincing evidence that recovery has commenced. Under the simple assumption of linear density dependence, rapid population growth is predicted. That this has not transpired leads to consideration of less simple assumptions regarding density dependence, such as depensation.

Résumé : Après sept ans de moratoire sur la pêche de la morue du Nord, nous n'avons aucune preuve convaincante d'un début de rétablissement. Sur la base d'une simple hypothèse de dépendance linéaire à l'égard de la densité, on prévoit une croissance rapide de la population. Le fait que cela ne se soit pas vérifié appelle à envisager des hypothèses plus complexes quant à la dépendance à l'égard de la densité, et notamment la dépensation.

[Traduit par la Rédaction]

Simple compensatory models of collapse and recovery

The underlying model that is generally assumed in fisheries assessments is decreasing recruits per unit spawner biomass with increasing spawner biomass. This leads to a spawner stock–recruit function in which recruitment initially increases with increasing spawner biomass and then either reaches an asymptote (Beverton–Holt model) or reaches a peak and then declines (Ricker model). Declining per capita reproductive success with increasing stock size, characteristic of both Beverton–Holt and Ricker models, is termed a "compensatory" response.

The amount of recruitment required to replace the spawner biomass that gave rise to it depends on the postrecruit mortality rate and weights and proportion mature at age. Where these quantities remain constant over time, replacement recruitment can be plotted as a straight line through the origin in the stock–recruit plot, the slope being determined by the postrecruit mortality rate, weight, and maturity values. For constant maturity and weight at age (common assumptions), the slope of the line depends only on postrecruit mortality rate, steeper slopes being associated with higher mortality. When recruitment exceeds the amount required to replace the spawner biomass, the population will increase, whereas when it falls below replacement, the population will decline. Equilibrium points occur where the replacement line intersects the recruitment curve. There is a single, nonzero, stable equilibrium point for the Beverton–Holt and Ricker models at moderate exploitation levels.

Based on these simple models, it can be predicted that overharvested stocks will recover to a stable equilibrium at higher spawner population size when fishing mortality is removed, since the slope of the replacement line decreases and intersection with the recruitment curve occurs at a higher spawner biomass. The northern cod (*Gadus morhua*) stock off the east coasts of southern Labrador and Newfoundland has been under a moratorium on commercial fishing since July 1992. However, in the most recent assessment (Lilly et al. 1999), 7 years later, there is no convincing evidence that recovery has commenced.

Depensation and the predator pit

One explanation for lack of recovery of northern cod is that per capita reproductive success (recruits per unit spawner biomass) has declined, perhaps as a result of increased predation on prerecruit fish. When reproductive success declines with decreasing population size, this is termed "depensation." Depensation can cause a second, lower equilibrium point to exist, depending on the slope of the replacement line. This equilibrium point is unstable: any variation in recruitment or spawning stock biomass will cause the population to either grow towards the upper, stable equilibrium point or to collapse to zero. Existence of depensation could therefore slow recovery.

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If the number of recruits per spawner decreases over some range of low stock sizes, but increases again at even lower stock size, then the potential arises for a second stable equilibrium point to occur where the replacement line intersects the recruitment curve. This "predator pit" phenomenon (Walters 1986, p. 83) could occur in cod if, for example, predator abundance and predation rates on prerecruits do not initially decrease with decreasing cod abundance, but predators switch to alternative prey at some low level of cod abundance. The predator pit provides a mechanism for prolonged low abundance even after fishing mortality has been removed. Recovery would require one or more year-classes that are strong enough to allow the population to escape the predator pit. If mortality is reduced, the slope of the replacement line decreases, increasing the probability of recovery.

Is depensation common in fish stocks?

We are unaware of any attempt to statistically examine stock–recruit data for the predator pit model. However, Myers et al. (1995*a*) analyzed spawner stock–recruit data for 128 fish stocks (Myers et al. 1995*b*) for which there were 15 or more years of data to determine whether there was evidence that depensation existed in a significant number of stocks. Only three of 128 stocks showed statistically significant evidence of depensation at the 0.05 level. Through an analysis of statistical power, Myers et al. (1995*a*) concluded that, if depensation did in fact occur in these stocks, more than three of 128 stocks would be expected to have indicated significant depensation. On the basis of the large sample size, they concluded that depensation does not generally occur in fish stocks and, consequently, the effects of overfishing are reversible. In the absence of depensation, Myers et al. (1997) predicted an annual growth rate of 19% for northern cod following collapse. After 7 years of moratorium on commercial fishing, the stock should have more than tripled in size.

Myers et al.'s (1995*a*) power analysis

Leaving aside the more difficult (but perhaps more relevant) problem of evaluating the applicability of the predator pit model, we examined the analysis of statistical power performed by Myers et al. (1995*a*) and found that, while having the advantage of being empirically based, it is complicated and leads to an inappropriate estimate of power. We carried out an analysis which, although not empirically based, is more defendable and, from the results, contend that it would be difficult to detect significant depensation at the 0.05 level for most cod populations.

In the analysis by Myers et al. (1995*a*), the model fitted to the data is the depensatory form of the Beverton–Holt model:

$$R = \frac{\alpha S^{\delta}}{1 + (S^{\delta}/K)}$$

where R is recruitment, S is spawner biomass, α is the slope of the recruitment curve at $S = 0$, αK is the asymptote, and δ is the depensation parameter. When $\delta = 1$, there is no depensation; depensation occurs for $\delta > 1$. Myers et al. (1995*a*) assumed a lognormal distribution (shape parameter σ) of residual variability caused by natural variation and measurement error. Myers et al. (1995*a*) obtained maximum likelihood estimates of model parameters with $\delta = 1$ and δ free and performed a likelihood ratio test to determine the significance of improvement in fit for δ free. For nine of the 128 stocks, the model with δ as a free parameter gave a significantly better fit at the 0.05 level, but in only three of these cases was δ significantly greater than 1.

To carry out their power analysis, Myers et al. (1995*a*) first estimated α and K with $\delta = 1$ under the constraint that asymptotic recruitment could be no greater than maximum observed recruitment (we call this the constrained nondepensatory Beverton–Holt model) for each of the 128 stocks. They then set $\delta = 2$ and constructed (as opposed to fitted) a depensatory model for each stock that matched the constrained nondepensatory Beverton–Holt model at both 50% asymptotic recruitment and asymptotic recruitment (we call this the constructed depensatory model). We denote the parameters (α and K) of the fitted constrained nondepensatory Beverton–Holt model with subscript "f" and those of the constructed depensatory model with subscript "c." Applying the two constraints required for the constructed depensatory model, one obtains

$$R = \frac{\alpha_c S^{\delta}}{1 + (S^{\delta}/K_c)}$$

where $\alpha_c = \dfrac{\alpha_f}{K_f^{\delta - 1}}$ and $K_c = K_f^{\delta}$.

Myers et al. (1995*a*) generated fake recruitment values at each of the observed spawner biomass values for each stock from a lognormal distribution with shape parameter σ estimated from the fitted constrained nondepensatory Beverton–Holt model and mean from the constructed depensatory model. They then fitted nondepensatory and depensatory Beverton–Holt models to the simulated data, as was done initially on the real data, and performed the likelihood ratio test for depensation. The model fits and likelihood ratio test were repeated on 100 sets of simulated recruitment data generated for each stock to estimate the statistical power. They found that at the 0.05 significance level, statistical power for detecting $\delta > 1$ was greater than 0.95 for 26 of 128 stocks for which data were simulated. Based on this result, they argued that if depensation is a general phenomenon in fish populations, they would have expected more than three of the 128 stocks examined to show significant depensation.

We applied the Myers et al. (1995*a*) approach to a hypothetical case. We arbitrarily selected $\alpha = 5$, $K = 200$, $\sigma = 0.6$, and $\delta = 2$ to give a depensatory Beverton–Holt stock recruit function from which 30 fake recruitment data points were generated for a range of spawner biomass values selected randomly from a uniform distribution between zero and the spawner biomass corresponding to 95% of the asymptotic recruitment (Fig. 1A). We then compared the fit of depensatory and nondepensatory Beverton–Holt models to the data using a likelihood ratio test and found that the depensatory model did not provide a significantly better fit at the 0.05 level. A constrained nondepensatory Beverton–Holt model was then fit to the data generated from this curve. Using the estimated parameters of this function,

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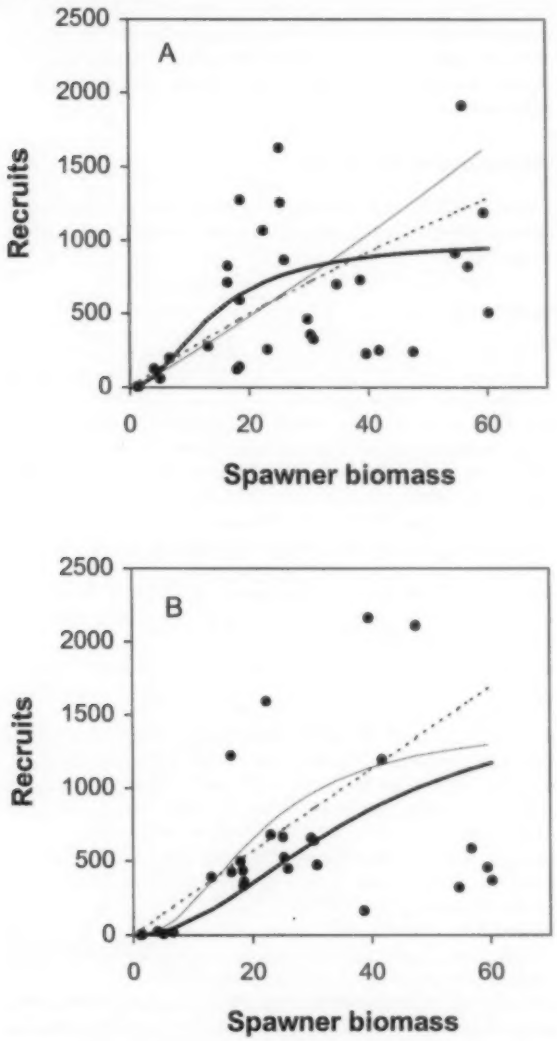
Fig. 1. (A) Depensatory Beverton–Holt stock–recruit function (bold solid line) defined by arbitrarily selected parameters $\alpha = 5$, $K = 200$, $\sigma = 0.6$, and $\delta = 2$ from which 30 data points were randomly generated. Fits of nondepensatory (light broken line) and depensatory (light solid line) models are compared. (B) Constructed depensatory model (bold solid line) based on the data in Fig. 1A (see text for details) from which a second set of 30 recruit data points were randomly generated. Fits of nondepensatory (light broken line) and depensatory (light solid line) are compared.



we followed the approach of Myers et al. (1995*a*) and constructed a depensatory model from which a second set of 30 fake recruitment values were generated, corresponding to spawner biomass values as in Fig. 1A, from a lognormal distribution with σ estimated from the fitted constrained nondepensatory model (Fig. 1B). We then compared the fit of the nondepensatory and depensatory Beverton–Holt models to the data generated from the constructed depensatory model and found that the depensatory model provided a significantly better fit at the 0.05 level. We repeated this exercise 100 times, and in 95% of the cases where the depensatory model did not provide a significant fit to the original data, a significant fit was obtained for a depensatory model applied to the data generated from the constructed depensatory model. This general outcome was robust to a wide range of alternative parameter combinations, number of data points, and range and scatter of spawner biomass values. Thus, we concluded that the arbitrary choice of the form of the constructed depensatory model in the Myers et al. (1995*a*) study exaggerates the power of detecting depensation. Other choices may have led to lower estimates of power.

An alternative analysis

We carried out an alternative, less complicated power analysis that, in general terms, follows an established Monte Carlo approach (Peterman and Routledge 1983; Peterman and Bradford 1987; Peterman 1990). The model used to generate the fake data and the model for which the power is being evaluated are, unlike the Myers et al. (1995*a*) study, identical. The depensatory Beverton–Holt model was used to generate fake data. The fits of the depensatory Beverton–Holt model and the nondepensatory Beverton–Holt model were then compared. The model that we used to generate fake data was constrained to have the asymptote αK constant. To explore the effect of obtaining data scattered across different ranges of spawner biomass, the upper limit on the recruitment axis from which fake data were generated was set to a predetermined percentage of asymptotic recruitment. This was accomplished by restricting expected recruitment to $\left[0, \left(\frac{\gamma}{\gamma+1}\right)\alpha K\right]$ and adjusting γ to explore various ranges of asymptotic recruitment. Since the range of recruitment values is fixed, adjusting the parameters of the Beverton–Holt model (α and K with αK constant) will simply rescale the spawner biomass axis. By scaling the spawner biomass axis using a factor of $(\gamma K)^{1/\delta}$, power need only be determined for different combinations of δ and σ and for a range of data set sizes variously scattered on the spawner biomass axis.

For each simulation, we generated pseudorandom fake recruitment data (assuming lognormal error about the mean value predicted by the depensatory Beverton–Holt model) at randomly selected (uniformly distributed) spawner biomass values between zero and the predetermined maximum spawner biomass chosen for the trial. Nondepensatory and depensatory Beverton–Holt models were then fit to the data and the likelihood ratio test applied at the 0.05 significance level. This procedure was repeated 100 times for each trial, and 0.95 power isopleths were mapped corresponding to the 0.05 significance level. The isopleths for trials consisting of combinations of 30 and 60 years of data and exploring 90, 95, or 98% of asymptotic recruitment are plotted in σ–δ parameter space as examples (Fig. 2). Because of random variability within each simulation, the 0.95 power isopleths were approximated by applying a quadratic smoother to points in the range 0.93–0.97.

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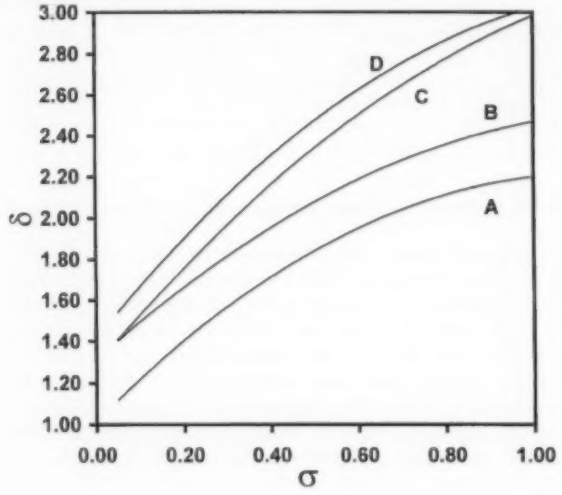
Fig. 2. Simulated approximate 0.95 power isopleths for detecting depensation at the 0.05 significance level in σ–δ parameter space for example trials: A, 60 years of stock–recruit data with spawner biomass range from zero to a spawner biomass corresponding to 90% of asymptotic recruitment; B, 30 years of data, 90% of asymptotic recruitment; C, 30 years of data, 95% of asymptotic recruitment; D, 30 years of data, 98% of asymptotic recruitment.



Depensation is difficult to detect and may be occurring in northern cod

Our results (Fig. 2) show that it will be hard to detect depensation unless δ is large, σ is small, the time series is long, and many data points are concentrated in the range of spawner biomass where depensation occurs (corresponding to a lower percentage of asymptotic recruitment in our analysis). Although our study lacks the empirical basis of the Myers et al. (1995*a*) study, our simulated power isopleths can be placed in an empirical context. For the 23 cod stocks illustrated in Myers et al. (1995*b*), 11 had data for more than 30 years, but for many of these, there is a paucity of data for spawner biomass levels where depensation might be expected to occur. Values of σ were generally high (often greater than 0.5, some greater than 0.8) for the non-depensatory Beverton–Holt model fit in these stocks. True values for δ are of course unknown.

If depensation is difficult to detect, it may not be as rare as previously suggested (also see Liermann and Hilborn 1997). Could it be occurring in northern cod? Recent data (Lilly et al. 1999) do not allow a formal test for depensation because bottom trawl surveys do not appear to be representative of the remaining fish, and accurate model estimates of population numbers at age have not been achieved. However, the remaining fish (upper limit estimated to be 150 000 t) occur in high densities in a few localities in the inshore. The harp seal population was estimated to be about 4.8 million in 1994 and growing at about 5% per year (Shelton et al. 1996). Stenson et al. (1997) estimated that in 1994, harp seals were consuming about 88 000 t of northern cod, mainly prerecruits. The proportion of cod in the nearshore diet of harp seals, although small, has not declined in recent years (G. Stenson, Department of Fisheries and Oceans, St. John's, Nfld., personal communication). These conditions are conducive to depensation. However, other explanations for apparent lack of recovery of northern cod under the moratorium also need to be given consideration, for example, loss of genetic components, continuing fishing activity, and environmental influences.

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References

Liermann, M., and Hilborn, R. 1997. Depensation in fish stocks: a hierarchic Bayesian meta-analysis. Can. J. Fish. Aquat. Sci. **54**: 1976–1984.

Lilly, G.R., Shelton, P.A., Brattey, J., Cadigan, N., Murphy, E.F., and Stansbury, D.E. 1999. An assessment of the cod stock in NAFO Divisions 2J+3KL. DFO Can. Stock Assess. Secetariat Res. Doc. 99/42.

Myers, R.A., Barrowman, N.J., Hutchings, J.A., and Rosenberg, A.A. 1995*a*. Population dynamics of exploited fish stocks at low population levels. Science (Washington, D.C.), **269**: 1106–1108.

Myers, R.A., Bridson, J., and Barrowman, N.J. 1995*b*. Summary of worldwide stock and recruitment data. Can. Tech. Rep. Fish. Aquat. Sci. No. 2024.

Myers, R.A., Mertz, G., and Fowlow, P.S. 1997. Maximum population growth rates and recovery times for Atlantic cod, *Gadus morhua*. Fish. Bull. U.S. **95**: 762–772.

Peterman, R.M. 1990. Statistical power analysis can improve fisheries research and management. Can. J. Fish. Aquat. Sci. **47**: 2–15.

Peterman, R.M., and Bradford, M.J. 1987. Statistical power of trends in fish abundance. Can. J. Fish. Aquat. Sci. **44**: 1879–1889.

Peterman, R.M., and Routledge, R.D. 1983. Experimental management of Oregon coho salmon (*Oncorhynchus kisutch*). Can. J. Fish. Aquat. Sci. **40**: 1212–1223.

Shelton, P.A., Stenson, G.B., Sjare, B., and Warren, W.G. 1996. Model estimates of harp seal numbers-at-age for the Northwest Atlantic. NAFO Sci. Counc. Stud. **26**: 1–14.

Stenson, G.B., Hammill, M.O., and J.W. Lawson. 1997. Predation by harp seals in Atlantic Canada: preliminary consumption estimates for Arctic cod, capelin and Atlantic cod. J. Northwest Atl. Fish. Sci. **22**: 137–154.

Walters, C.J. 1986. Adaptive management of renewable resources. Macmillan Publishing Co., New York.

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Impacts of atypical data on Bayesian inference and robust Bayesian approach in fisheries

Y. Chen and D. Fournier

Abstract: Bayesian inference is increasingly used in fisheries. In formulating likelihood functions in Bayesian inference, data have been analyzed as if they are normally, identically, and independently distributed. It has come to be believed that the first two of the assumptions are frequently inappropriate in fisheries studies. In fact, data distributions are likely to be leptokurtic and (or) contaminated by occasional bad values giving rise to outliers in many fisheries studies. Despite the likelihood of having outliers in fisheries studies, the impacts of outliers on Bayesian inference have received little attention. In this study, using a simple growth model as an example, we evaluate the impacts of outliers on the derivation of posterior distributions in Bayesian analyses. Posterior distributions derived from the Bayesian method commonly used in fisheries are found to be sensitive to outliers. The distributions are severely biased in the presence of atypical values. The sensitivity of normality-based Bayesian analyses on atypical data may result from small "tails" of normal distribution so that the probability of occurrence of an event drops off quickly as one moves away from the mean a distance of a few standard deviations. A robust Bayesian method can be derived by including a mixture distribution that increases the size of tail so that the probability of occurrence of an event does not drop off too quickly as one moves away from the mean. The posterior distributions derived from this proposed approach are found to be robust to atypical data in this study. The proposed approach offers a potentially useful addition to Bayesian methods used in fisheries.

Résumé : L'inférence bayesienne est de plus en plus utilisée dans les sciences halieutiques. Pour formuler des fonctions de vraisemblance dans le cadre de l'inférence bayesienne, on a analysé les données comme si elles étaient distribuées de façon normale, identique et indépendante. On en est venu à croire que pour les études halieutiques, il ne convient souvent pas de considérer les données comme étant distribuées de façon normale et identique. De fait, les distributions de données sont probablement leptocurtiques et (ou) contaminées par de mauvaises valeurs occasionnelles qui font apparaître des valeurs aberrantes dans bon nombre d'études halieutiques. Malgré le fait qu'il est probable de voir apparaître des valeurs aberrantes dans les études halieutiques, les impacts de ces valeurs sur l'inférence bayesienne ont été peu étudiés. Dans la présente étude, qui fait appel à un modèle de croissance simple à titre d'exemple, nous évaluons les impacts des valeurs aberrantes sur l'établissement de distributions a posteriori dans des analyses bayesiennes. Les distributions a posteriori dérivées de la méthode bayesienne utilisée communément dans les pêches sont sensibles aux valeurs aberrantes. Les distributions se trouvent gravement biaisées par la présence de valeurs atypiques. La sensibilité des analyses bayesiennes fondées sur la normalité aux valeurs atypiques peut résulter de la présence de « queues » courtes dans la distribution normale, de sorte que la probabilité d'occurrence d'un événement chute rapidement quand on s'éloigne de la moyenne de seulement quelques écarts-types. On peut obtenir une méthode bayesienne robuste en intégrant une distribution composite, laquelle accroît la longueur des queues, ce qui fait que la probabilité d'occurrence d'un événement ne chute pas trop rapidement quand on s'éloigne de la moyenne. Les distributions a posteriori issues de l'approche proposée ici s'avèrent robustes à l'égard des données atypiques dans la présente étude. L'approche proposée constitue un complément potentiellement utile aux méthodes bayesiennes utilisées dans les sciences halieutiques.

[Traduit par la Rédaction]

Introduction

Mathematical models are commonly used to describe fisheries data (Hilborn and Walters 1992; Deriso and Quinn 1997). To relate a model to data observed in a fishery, an appropriate method is required to estimate parameters in the model. In general, there are two statistical approaches that can be used for parameter estimation: frequentist and Bayesian approaches. The statistical problem is similar for these two approaches: both are used to make statistical inferences about unknown parameters in the model (Berger 1985; Box and Tiao 1992).

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Frequentist inference is commonly used in fisheries studies (Hilborn and Walters 1992). It assumes that parameters being estimated are fixed constant and data are random observations from some unknown statistical population (Cox and Hinkley 1974; Ellison 1996). An objective function needs to be defined based on assumptions made on random variables (Hilborn and Walters 1992; Chen and Paloheimo 1998). Parameters and their confidence intervals can then be estimated by optimizing the objective function (Fournier and Archibald 1982; Deriso et al. 1985).

Bayesian inference has been used increasingly in fisheries (Hilborn and Walters 1992; Hilborn et al. 1993; Walters and Ludwig 1994; Kinas 1996; Walters 1998). This approach assumes that parameters are random as opposed to constant for frequentist inference. Instead of estimating the "true" values of the parameters as in frequentist inference, it only looks at the statistical distributions of the values of the parameters (Cox and Hinkley 1974; Ellison 1996).

Bayesian methods use a probability rule (Bayes' theorem) to calculate a "posterior distribution" from the observed data and a "prior distribution," which summarizes the prior knowledge of the parameters (Dennis 1996; Taylor et al. 1996). Bayes' theorem states that the probability of parameter β given the data x, $p(\beta|x)$, is proportional to the product of the probability of the data x given parameter β, $p(x|\beta)$, and the probability of the parameter, $p(\beta)$, not conditioned upon the data. The probability $p(\beta|x)$ is the posterior distribution for parameter β and is the result of the analysis. The probability $p(x|\beta)$ is called the likelihood function and can also be written as $L(\beta|x)$. The probability $p(\beta)$ is the prior distribution for β and represents the probability distribution for β before data x are known. Thus, the posterior distribution is equal to the product of the likelihood function and the prior distribution, normalized by the integral of the product of the likelihood and the prior:

$$(1) \qquad p(\beta|x) = \frac{L(\beta|x)p(\beta)}{\int L(\beta|x)p(\beta)d\beta}.$$

For many fisheries studies, Bayesian inference is perhaps more appropriate than frequentist inference in that it can incorporate the prior knowledge on fisheries into parameter estimation (Hilborn and Walters 1992; Walters 1998). Such knowledge existing in most fisheries can be obtained from biological and ecological theories, experience from other fisheries, fishers' experience, and scientists' insights into the fisheries being studied.

The use of likelihood functions in Bayesian inference makes it easy to incorporate data of various sources and uncertainties associated with the data (Taylor et al. 1996). Uncertainties associated with fisheries stock assessment often make decisions about management strategies difficult (Kinas 1996). Bayesian inference provides a systematic approach that explicitally incorporates both uncertainties and risks in analyses. This is essential to improve fisheries management (Hilborn et al. 1993). It has been predicted that Bayesian inference will be used in most fish stock assessment studies in the near future (Hilborn and Walters 1992).

Fisheries data have frequently been analyzed as if, to an adequate approximation, errors are normally, identically, and independently distributed. It has come to be believed that the first two of the assumptions are frequently inappropriate in fisheries studies (Hilborn and Walters 1992; Chen et al. 1994). In fact, error distributions are likely to be leptokurtic and (or) contaminated by occasional bad values giving rise to outliers in many fisheries studies (Chen et al. 1994).

Errors inherent in fisheries studies can be categorized into four key forms: measurement errors, process errors, model errors, and operating errors (Chen and Paloheimo 1998). Measurement error results from our inability to measure fisheries data perfectly, and process error results from our inability to describe fisheries process perfectly. Model error is due to ignorance about the appropriate model to represent complex fisheries process, and operating error may occur when the control exerted on a fish population is not the one that is observed or expected (e.g., measured fishing effort differs from effective fishing effort, which is proportional to fishing mortality rate; Chen and Paloheimo 1998). These errors are usually assumed to follow certain statistical distributions (e.g., normal, lognormal; Fournier and Archibald 1982; Hilborn and Walters 1992), although such assumptions may not be realistic in many fisheries studies (Polacheck et al. 1993; Walters 1998). Commonly used frequentist methods for parameter estimation, such as least squares and maximum likelihood estimators, are usually not robust to atypical observations and assumptions concerning errors, and their estimated parameters can be grossly biased if there are atypical data and (or) an unrealistic assumption is made with respect to the error structure in modeling fisheries data (Schnute 1989; Chen et al. 1994; Chen and Andrew 1998). Many methods that are robust to outliers and (or) assumptions on errors are available for frequentist inference (e.g., Rousseeuw and Leroy 1987; Lawrence and Arthur 1990).

Because of the likelihood of having outliers in fisheries studies, it is necessary to evaluate impacts of outliers on the performance of an estimator and desirable to apply an estimator that is robust to outliers in fisheries studies. The impacts of outliers on parameter estimation using the frequentist approach have been studied in fisheries (Chen et al. 1994). However, the possible impacts of outliers on the derivation of posterior distributions in Bayesian analyses have received little attention in fisheries.

The objectives of this study are to evaluate whether and how outliers may affect posterior distributions calculated from the Bayesian method commonly used in fisheries and to propose a general approach that can be used to reduce the sensitivity of posterior distributions to outliers. Limited by the number of fisheries models that can be included, we used a simple but commonly used growth model, the von Bertalanffy growth function (Ricker 1975), in this study as an example. This model was used to describe the size-at-age data for white sucker (*Catostomus commersoni*) sampled from Dickie Lake in Ontario (Chen 1991). Three sets of data were included in the study. The first set comprises the original data having no outliers (Fig. 1). The second set of data was simulated from the first one by creating one atypical data point. The third set of data was simulated from the original one by creating two atypical data points. Impacts of outliers on the derivation of posterior distributions were evaluated by comparing results derived from the three data sets. The proposed two-component mixture distribution function was also included in the study. The robustness of

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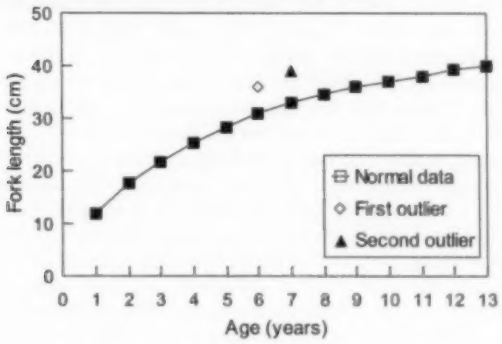
Fig. 1. Length-at-age data for female white sucker in Dickie Lake, Ontario. Data were taken from Chen (1991).



this proposed Bayesian approach to outliers in deriving posterior distributions was evaluated. It should be noted, however, that this study is only intended for proposing a general approach, which is based on a mixture distribution function, toward developing a robust Bayesian approach and that only one model and one type of mixture distribution function were included in the study. The performance of different mixture distribution functions with different fisheries models should be evaluated using simulation studies similar to the one presented in this study.

Likelihood function for Bayesian analysis

Assume that data obtained in a fisheries study can be defined by the following model:

$$(2) \qquad Y = f(X_1,..., X_k; \beta_1,..., \beta_J) + \varepsilon$$

where Y is the dependent variable, $X_1,..., X_k$ are the independent variables, $\beta_1,..., \beta_J$ are the parameters in the model, and ε is an error term assumed to follow the normal distribution $N(0, \sigma^2 I)$. The likelihood function of the data, given β, can be written as

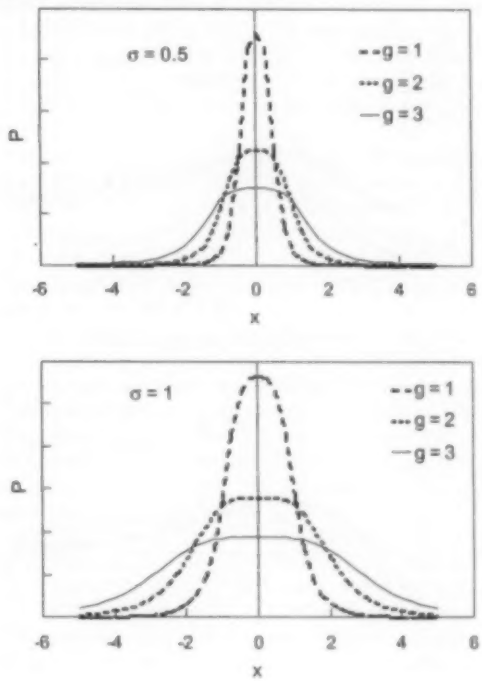
$$(3) \quad L(\beta|X,Y) = \prod_{i=1}^{N} \left\{ \frac{1}{\sqrt{2\pi}\sigma} \exp\left[-\frac{(Y_i - f_i(X_1,..., X_k;\beta))^2}{2\sigma^2} \right] \right\}$$

where N is the number of observations.

Bayesian inference based on eq. 3 tends to be sensitive to outliers. The reason is simple: a normal distribution has a small "tail" so that the probability of occurrence of an event drops off quickly as one moves away from the mean a distance of a few standard deviations. To develop a robust Bayesian method that is less sensitive to outliers, but also performs well in the absence of the outliers, we need to use a mixture distribution that can increase the size of tail so that the probability of occurrence of an event does not drop off too quickly as one moves away from the mean. This can be achieved by using a mixture distribution that assumes that errors are either $N(0, \sigma^2)$ with probability $1 - p$ or a fat-tailed distribution with probability p.

There are many probability density functions that can be used as the fat-tail distribution in a mixture distribution. In this study, we propose that the probability density function of the fat-tailed distribution be given by

Fig. 2. Fat-tailed probability distributions defined by eq. 4 with different values of g that determine the sizes of tails of the distribution for two levels of variance σ.



$$(4) \qquad P(x) = \frac{2}{\sqrt{\pi g}\sigma}\left[1 + \frac{x^4}{(g\sigma)^4}\right]^{-1}$$

where the parameter g can be used to adjust the spread of the fat-tailed distribution (Fournier 1996). An increase in the value of g tends to increase the "thickness" of the tails, thus increasing the probability of having extreme values that are far away from the mean value (Fig. 2).

For the proposed two-component mixture distribution, the normal distribution, having a large a prior probability (i.e., $1 - p$), represents the "true" population of observations, and the fat-tailed distribution, having a small a prior probability (i.e., p), represents the "problem" observations (i.e., outliers). The value of p was set at 0.05 in this study. The corresponding likelihood function for observations is

$$(5) \quad L(\beta, \sigma|X,Y) = \prod_{i=1}^{N} \left\{ \frac{1-p}{\sqrt{2\pi}\sigma} \exp\left[-\frac{(Y_i - f_i(X_1,..., X_k;\beta))^2}{2\sigma^2} \right] + \frac{2p}{\sqrt{\pi g}\sigma}\left[1 + \frac{(Y_i - f_i(X_1,..., X_k;\beta))^4}{(g\sigma)^4}\right]^{-1} \right\}.$$

The $L(\beta|x)$ in eq. 1 can be replaced with eq. 5. Given a prior for parameter β, we are able to derive the posterior distribution for β, $p(\beta|x)$, from eq. 1.

The probability distribution described by the proposed mixture distribution is influenced by the values of p and g. The value of p, which represents the proportion of data that

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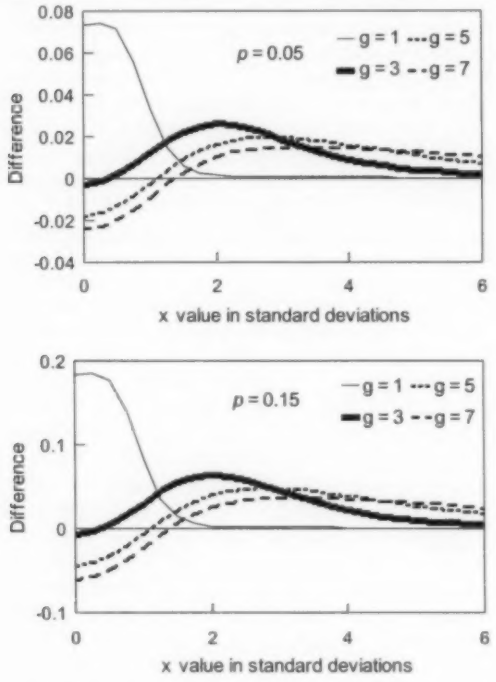
Fig. 3. Impacts of different g and p values on the mixture probability distribution. The difference is calculated by subtracting the probability of the normal distribution from the probability of the two-component mixture distribution.



are contaminated by abnormal errors, determines the relative importance of the two density distribution functions in describing the data. An increase in the p value implies an increase in the weight of the fat-tailed function in deriving the posterior distribution. If we know the number of atypical values in a data set, the value of p can be determined readily. In practice, however, it is unlikely that we know the true value of p except that p must have a value between 0 (no outlier) and 0.5 (half of the data are outliers). Thus, the choice of the p value can only reflect our belief about the quality of data. This belief may come from our experience and understanding of the problem and data collection process in analyzing similar types of data.

The value of g in eq. 5 determines the probability of how far the atypical data may be away from the mean value. The choice of g values can be determined by our understanding of how far atypical data may distribute away from the mean. To illustrate the impacts of g values on the mixture probability distribution, we plotted the differences between the proposed two-component mixture probability distribution (eq. 5) and the normal probability distribution (eq. 3) for different g values (Fig. 3). The difference (i.e., y-axis in Fig. 3) was calculated as

$$\text{difference} = \frac{1-p}{\sqrt{2\pi}\sigma}\exp\left(-\frac{x^2}{2\sigma^2}\right) + \frac{2p}{\sqrt{\pi g}\sigma}\left[1+\frac{x^4}{(g\sigma)^4}\right]^{-1} - \frac{1}{\sqrt{2\pi}\sigma}\exp\left(-\frac{x^2}{2\sigma^2}\right).$$

Because the distributions are symmetric, only the right portions of the differences in the two distributions were presented. When g is 1, the mixture distribution function has larger values than the normal distribution, but such a difference decreases quickly when x moves away from the mean. After moving 2 standard deviations away from the mean, the mixture distribution function has probability values that are virtually the same as for the normal distribution (Fig. 3). Because an atypical datum is unlikely to have a value within 2 standard deviations from the mean (atypical data must have a value far away from the mean), 1 is not likely an optimal value for g. When g is 3, the probability yielded by the mixture distribution is still larger than that of the normal distribution. However, such a difference becomes larger first when moving away from the mean and then decreases gradually. Similar patterns can be observed when g is 5 and 7. However, for these two values, the mixture distribution tends to have smaller probabilities of having values within one standard deviation compared with the normal distribution (Fig. 3). When g is 3, the mixture distribution has the highest probability between 1.5 and 3 standard deviations. This suggests that 3 is an appropriate value if atypical data lie within 2 and 3 standard deviations away from the mean. If atypical data are likely to have values between 3 and 4 standard deviations away from the mean, 5 is an appropriate value for g in eq. 5 (Fig. 3). In fisheries, we believe that an atypical datum that is difficult to identify without a detailed analysis is likely to fall within 4 standard deviations from the mean. Those data that lie beyond 4 standard deviations are rather obvious to identify. Thus, it may be appropriate to choose a g value between 3 and 5 in fisheries studies.

The combined impacts of different values of g and p on the mixture probability distribution are illustrated in Fig. 3. The relationships of the distance to mean and differences between the mixture and normal distributions for different g values are not changed with p values. The p values only change the scale of the differences between the mixture and normal probability distributions.

Smith and Gelfand (1992) suggested that an algorithm referred to as sampling–importance–resampling (Rubin 1988) is a particularly useful and simple integration technique for Bayesian statistics. In this approach, values for the parameters are randomly selected from their joint prior distribution to form a sample set β (a vector). The likelihood of the data, given this particular β, is calculated and stored. This procedure is repeated n_1 times, generating n_1 βs and their associated likelihoods. These n_1 βs are then resampled n_2 times with replacement, with probability equal to weight q, where

$$q_j = \frac{L_j(\beta|x)}{\sum_{j=1}^{n_1} L_j(\beta|x)}.$$

The generated distribution of size n_2 can be used to approximate the posterior distribution. Using the sampling–importance–resampling algorithm (Rubin 1988) with the likelihood defined in eqs. 3 and 5, we can estimate the posterior distribution for the commonly used and proposed Bayesian methods, respectively.

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Application

The impacts of outliers on Bayesian inference were illustrated with an example of modeling fish size-at-age data in this study. Size-at-age data were described by the commonly used von Bertalanffy growth function, written as

$$L_t = L_\infty(1 - e^{-K(t-t_0)}) + \varepsilon_t$$

where L_t is the size at age t, L_∞ is the maximum attainable length, K is the Brody growth parameter, t_0 is the age at the size of 0, and ε_t is an error term (Ricker 1975). The size-at-age data used in this study were taken from Chen (1991) for white sucker in Dickie Lake in Ontario (Fig. 1). There are 13 age groups in the data. The reasons for using this type of model and data in this study are simplicity of the model and visibility of outliers in plotting size-at-age data.

We first evaluated the impacts of having one outlier in the data on the derivation of posterior distributions if the commonly used Bayesian method (hereafter referred to as CBM), which is based on eq. 3 for the formulation of its likelihood function, was employed. An outlier was created by changing the size at age 6 from 30.9 to 35.9 cm while keeping the rest of the data unchanged (Fig. 1). The CBM was applied to this altered data set (i.e., data with an outlier) as well as to the original data set (i.e., data without an outlier). The differences in the derived posterior distribution for a parameter between these two data sets were evaluated using a comparison index (CI) defined as follows:

$$(6) \qquad CI = \sum_{i}^{n} [P(i) - PT(i)]^2$$

where i is an index of interval used in grouping values of the parameter for calculating the posterior probability distribution of the parameter, n is the total number of the intervals for the parameter, and $PT(i)$ and $P(i)$ are the probabilities of the parameter in interval i calculated using the CBM for data without and with the outlier, respectively. The intervals used to group resultant parameters in this study were $0.025 \cdot year^{-1}$ for K, 1 cm for L_∞ and 0.2 year for t_0. The CI measures how severely the posterior distribution of a parameter estimated in the presence of outliers departs from the posterior distribution estimated for data without outliers. A larger value of CI indicates a greater difference in posterior distributions derived from data with and without outliers. This implies that the estimation method is sensitive to outliers in deriving posterior distributions.

We then evaluated the robustness of the proposed Bayesian method (hereafter referred to as PBM) to outliers. The PBM was applied to both the data sets. The posterior distributions derived for the two data sets using the PBM were compared with the respective posterior distributions derived for data without outliers using the CBM. The CI defined in eq. 6 was used for the comparisons. In this case, $PT(i)$ in eq. 6 represents the probability estimated using the CBM for data without outliers, and $P(i)$ represents the probability estimated using the PBM for data with or without outliers. A small value of CI for the posterior distribution of each parameter in such a comparison suggests that impacts of the outlier on the derivation of the posterior distribution is small and that the PBM is robust to outliers.

The second outlier was simulated by changing size at age 7 from 33 to 39 cm. This outlier together with the first one (i.e., size at age 6) allowed us to evaluate the impacts of multiple outliers on CBM and PBM. The choice of having both outliers much larger than their true values, rather than having one larger and one smaller, reflects our belief that such a pattern (i.e., both outliers on one side of the growth curve) has greater impacts on the derivation of posterior distributions.

Different values of p and g were used in the simulation to evaluate their impacts on the derivation of posterior distributions. We used two values for p (0.05 and 0.15) and three values for g (1, 3, and 5). The combination of two p values, three g values, and the existence of different numbers of outliers (0, 1, and 2) results in 18 data sets for the PBM and three data sets for the CBM.

To test whether different priors might affect the evaluation of the impacts of atypical data on Bayesian inference, we considered two scenarios in identifying priors for parameters. For the first scenario, we assumed that we did not know anything about the parameters except their possible ranges; for the second scenario, we had a prior knowledge about distributions of the parameters. For the first scenario, uniform distributions, often referred to as noninformative distributions in a Bayesian analysis of fisheries data, were assumed for all parameters. The following distributions were used for priors: $L_\infty \propto U(38, 58)$, $K \propto U(0, 1)$, $t_0 \propto U(-5, 3)$, and $\sigma \propto U(0.1, 10.1)$. The ranges of these distributions were large enough to cover all possible values that these parameters might have. For the second scenario, we assumed normal distributions for all parameters. Thus, informative priors were used in calculating posterior distributions. The following distributions were assumed for priors: $L_\infty \propto N(41.5, 5^2)$, $K \propto N(0.2, 0.1^2)$, $t_0 \propto N(-0.55, 0.1^2)$, and $\sigma \propto N(0.6, 0.3^2)$. The comparison of the resultant posterior distributions may shed light on the importance of priors in reducing the impacts of outliers on deriving posterior distributions.

Rubin's (1988) sampling–importance–resampling algorithm was used in deriving posterior distributions for the parameters. The first sample size n_1 was set at 2 000 000 and the resample size n_2 was set at 50 000. These numbers were derived from our preliminary study in which several different combinations of n_1 and n_2 were used. These numbers were found to be large enough to derive stable probability distributions for this study. They are also consistent with the n_1 and n_2 used in other studies (Taylor et al. 1996). Extensive computation is required, as one can expected with any Bayesian analysis. However, with easy access to powerful personal computers, this is not a problem. In this study, it took about 75 min to complete calculations for one data set on a Pentium I 233-MHz personal computer.

Results

Noninformative priors

Posterior distributions of the parameters derived for the altered data with the outlier(s) differed greatly from those derived for data without outlier(s) when the CBM was used. For each parameter, the CI value calculated for the CBM-estimated posterior distribution in the presence of outliers was much higher than those for the PBM (Table 1). The

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Table 1. Summary of the CI calculated using eq. 6 when priors were noninformative.

Data set	g	p	Method	Outliers	Parameter t_0	Parameter K	Parameter L_∞
I	na	na	CBM	0	0	0	0
II	na	na	CBM	1	1.08	0.84	1.01
III	na	na	CBM	2	0.96	0.88	1.29
IV	1	0.05	PBM	0	0.12	0.04	0.01
V	1	0.05	PBM	1	0.45	0.14	0.66
VI	1	0.05	PBM	2	0.89	0.75	0.80
VII	1	0.15	PBM	0	0.48	0.07	0.54
VIII	1	0.15	PBM	1	0.59	0.21	0.91
IX	1	0.15	PBM	2	0.89	0.71	0.74
X	3	0.05	PBM	0	0.01	0.01	0.45
XI	3	0.05	PBM	1	0.01	0.01	0.27
XII	3	0.05	PBM	2	0.33	0.16	0.36
XIII	3	0.15	PBM	0	0.28	0.01	0.48
XIV	3	0.15	PBM	1	0.17	0.01	0.38
XV	3	0.15	PBM	2	0.29	0.08	0.20
XVI	5	0.05	PBM	0	0.66	0.08	0.51
XVII	5	0.05	PBM	1	0.68	0.11	0.49
XVIII	5	0.05	PBM	2	0.87	0.26	0.56
XIX	5	0.15	PBM	0	0.85	0.11	0.52
XX	5	0.15	PBM	1	0.75	0.17	0.69
XXI	5	0.15	PBM	2	0.65	0.20	0.48

CI values tended to be smaller when there was only one outlier compared with those in the presence of two outliers. However, the difference was small (Table 1). Posterior distributions of three parameters were plotted for data sets I (no outlier) and II (one outlier) (Fig. 4). Modes of the distributions of all three parameters shifted to the right side after the outlier was introduced (Fig. 4). The shapes of the posterior distributions of the three parameters were also different for data with and without the outlier, with the posterior distributions derived for data with the outlier having longer tails than those for data without the outlier. Thus the CBM, which is commonly used in fisheries, was sensitive to outliers in deriving posterior distributions for model parameters.

When the PBM was used, small differences were observed in the posterior distributions derived for data with and without outliers. Differences in the CI values were small for the data sets with and without outliers when the PBM was used compared with those derived using the CBM (Table 1). The posterior distributions estimated using the PBM in the presence of outliers did not change much from those estimated without the outliers. This could be seen from the small differences in CI values when the PBM (with the same g and p values) was applied to data with and without outliers (Table 1). The choice of g and p values had large impacts on the CI values. The most appropriate value for g tended to be 3 in this study. This is consistent with our prediction that the value of g should be chosen based on how far outliers depart away from the mean (measured in standard deviations). The most appropriate p value seemed to be 0.05 when there was only one outlier. However, when there were two outliers, a p of 0.15 tended to result in smaller CI values. This is also consistent with our prediction that the value of p should be decided on by the proportion of data that are subject to atypical errors. In this study, one outlier represents 7.7% of the data, while two outliers represent 15% of the data. Overall, the PBM method with a g of 3 and a p of 0.05 had the smallest CI when there was one outlier, while the PBM with a g of 3 and a p of 0.15 had the smallest CI (Table 1). When these optimal values were not used, the PBM tended to yield large CI values (Table 1). For all values of g and p used in this study, the PBM had smaller CI values than the CBM (Table 1). This suggests that the inclusion of outliers has smaller impacts on the PBM-estimated posterior distributions compared with impacts on the CBM-estimated posterior distributions, even though the values of g and p are not optimal. Posterior distributions of the parameters derived using the PBM were plotted for data sets X (no outlier) and XI (one outlier) (Fig. 4). The PBM-estimated posterior distributions were similar to those estimated using the CBM for the data without the outlier (Fig. 4). The differences in the posterior distributions derived for data sets X (no outlier) and XI (one outlier) were small (Fig. 4). For parameters t_0 and K, the PBM-estimated posterior distributions were almost identical to those derived using the CBM for data without the outlier (Fig. 4). Thus, for the PBM, when an outlier was introduced into the data, the results in posterior distributions for parameters did not change greatly from those estimated in the absence of the outlier, and when there was no outlier, the differences in posterior distributions were small between the normal and proposed methods (Fig. 4). All the above results suggest the PBM to be much less sensitive to outliers than the CBM and the PBM to be robust to outliers in deriving posterior distributions in Bayesian analysis.

Posterior distributions of σ were plotted for data sets I, II, X, and XI (see Fig. 4). Large differences were observed in the posterior distributions of σ derived using the CBM for data with and without the outlier (Fig. 4). The CBM-derived posterior distribution of σ for data with the outlier shifted to the right of the CBM-derived posterior distribution of σ for data without the outlier, and there was almost no overlap between these two distributions (Fig. 4). For the PBM, the change in the posterior distributions of σ was small after the inclusion of the outlier (Fig. 4). The CBM-derived posterior distribution of σ for data without the outlier was found to be similar to those derived using the PBM.

Informative priors

The comparison results observed for informative priors were similar to those when priors were noninformative. The presence of outliers in the data resulted in large changes in the CBM-derived posterior distributions (see plots for data sets I and II in Fig. 5). However, such changes were smaller than those derived when priors were noninformative (Figs. 4 and 5). For all three parameters, the CI values of the CBM-derived posterior distributions for data with one or two outliers decreased substantially when priors were informative compared with when priors were noninformative (Tables 1 and 2). This may suggest that informative priors tend to reduce the sensitivity of the CBM to outliers in deriving posterior distributions. The PBM-derived posterior distributions still had small differences for data with and without outliers (see plots for data sets X and XI in Fig. 5). For all three parameters, the CI values of the PBM-derived posterior distributions were still smaller than those for the

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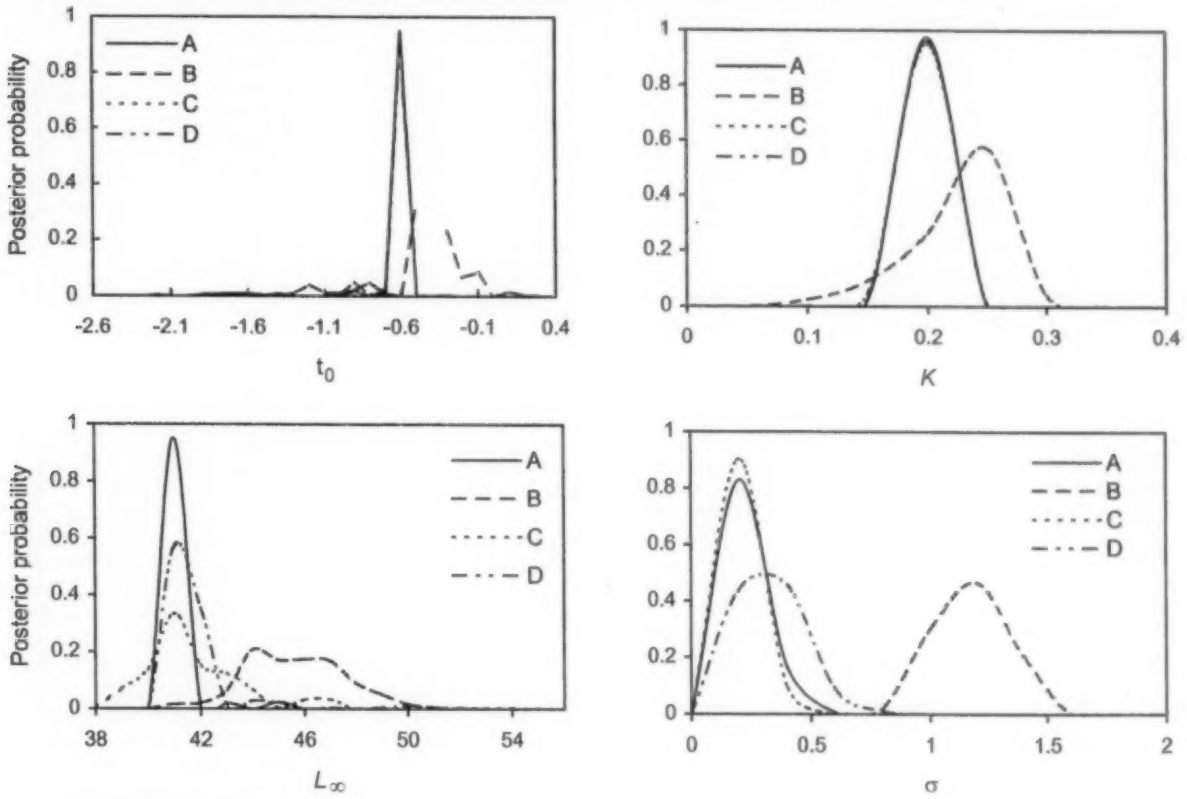
Fig. 4. Summary of the posterior distributions for parameters of the von Bertalanffy growth function derived using the CBM and PBM for data sets I, II, X, and XI (see Table 1) when priors were noninformative. A, posterior distributions derived using CBM in the absence of the outlier; B, posterior distributions derived using CBM in the presence of the outlier; C, posterior distributions derived using PBM in the absence of the outlier; D, posterior distributions derived using PBM in the presence of the outlier.



CBM-estimated posterior distributions when there was an outlier (Table 2). The impacts of g and p values on CI values were the similar to those when priors were noninformative. The PBM with a g of 3 and a p of 0.05 had the smallest CI when there was one outlier, while the PBM with a g of 3 and a p of 0.15 had the smallest CI (Table 2). It was clear that the PBM was still less sensitive than the CBM to outliers when priors were informative.

Results of comparisons between the two methods for posterior distributions of σ were similar to those when priors were noninformative (see plots for data sets I, II, X, and XI in Figs. 4 and 5). Differences observed in the posterior distributions of σ derived using the CBM for data with and without the outlier were large (Fig. 5). The CBM-derived posterior distribution of σ for data with the outlier tended to have larger values than that for data without the outlier. For the PBM, the change in the posterior distributions of σ was small after the inclusion of the outlier (Fig. 5). The CBM-derived posterior distribution of σ for data without the outlier was found to be similar to those of the PBM (Fig. 5).

Discussion

This study suggests that the Bayesian method commonly used in fisheries, which is based on eq. 3, is sensitive to outliers. The existence of outliers may result in large biases in derived posterior distributions. This study also suggests that the provision of informative priors for parameters to be estimated may reduce the severity of the biases caused by outliers. This provides fisheries scientists with a weapon in the fight of reducing negative impacts of outliers on parameter estimation using the CBM in fisheries studies. It indicates that the quality of the prior information about the studied fisheries is important to Bayesian inferences in overcoming problems in the quality of fisheries data.

The proposed Bayesian approach based on eq. 5 was found to be robust to outliers in this study. When there were no outliers, the posterior distributions derived from this approach were similar to those derived from the CBM. In the presence of outliers, the posterior distributions derived from this method changed little. Fisheries data tend to be subject to errors of many types (see Introduction), and it is likely that outliers exist in many fisheries studies (e.g., Chen et al. 1994). Because of the multivariate nature of fisheries data, it is often difficult to identify outliers. It is thus desirable to use a Bayesian method that is robust to outliers. In this case, the derivation of posterior distributions for model parameters will not be affected greatly by outliers. The proposed Bayesian approach has shown its robustness to outliers with respect to the derivation of posterior distributions in this

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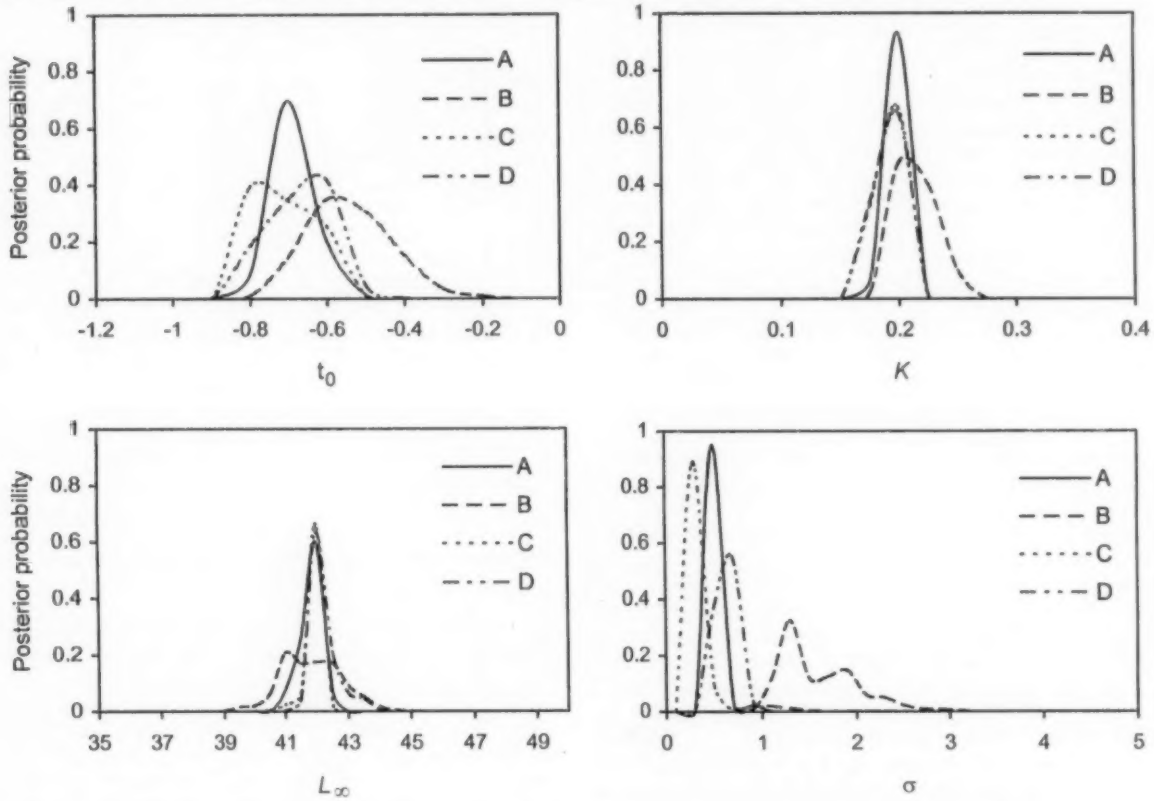
Fig. 5. Summary of the posterior distributions for parameters of the von Bertalanffy growth function derived using the CBM and PBM for data sets I, II, X, and XI (see Table 2) when priors were informative. A, posterior distributions derived using CBM in the absence of the outlier; B, posterior distributions derived using CBM in the presence of the outlier; C, posterior distributions derived using PBM in the absence of the outlier; D, posterior distributions derived using PBM in the presence of the outlier.



study. However, it should be pointed out that more substantial studies may be needed to further evaluate the performance of this approach.

A problem associated with applying the proposed approach in fisheries studies is to identify an appropriate value for g and p in the mixture distribution function (i.e., eq. 5). An optimal value for g and p can greatly improve the performance of the PBM. Although we know that g should have a value between 3 and 5 and that p should range between 0 and 0.5, the actual values for these two parameters may never be known in fisheries. Fisheries scientists should determine the values of g and p based on their understanding of the proportion of data that may be affected by abnormal errors and distributions of these atypical data. An alternative approach is to include g and p in the parameters to be estimated. However, this increases the number of parameters to be estimated. This study indicates that the PBM tends to outperform the CBM when outliers exist in the data, even though the values of g and p are not optimal (Tables 1 and 2). Thus, we will always better off using the PBM when outliers exist.

Small tails in normal probability distributions are identified as the cause for outliers arising in using the Bayesian method based on eq. 3. To make a Bayesian method robust, we need to increase the size of the tails in probability distributions. A two-component mixture probability distribution with one component being a normal density function and the other being a different function is surely an appropriate approach to achieving this goal. There are many probability functions that can be used in a mixture distribution together with the normal distribution to increase the size of the tails. This study explores only one of them (i.e., eq. 4). More studies can be done to explore other types of mixture distributions.

The information about posterior distributions obtained in Bayesian inferences is often used directly in managing fisheries resources (Walters 1998). For example, in fish stock assessment, the derived posterior distributions of some parameters that are vital in describing the dynamics of fish populations are often used directly for risk analysis with respect to certain management strategies (Walters and Ludwig 1994; Walters 1998). So far, no attention has been paid to the impacts of outliers on Bayesian analyses. This study shows that the existence of outliers may greatly bias the derived posterior distributions. The likelihood of having outliers in fisheries studies implies that posterior distributions derived from the Bayesian method based on eq. 3 in fisheries may be unreliable. This may lead to erroneous results on the dynamics of fish stocks and subsequently the adaptation of an inappropriate strategy in managing fisheries resources.

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Table 2. Summary of the CI calculated from eq. 6 when priors were informative.

Data set	g	p	Method	Outliers	Parameter t_0	Parameter K	Parameter L_∞
I	na	na	CBM	0	0	0	0
II	na	na	CBM	1	0.44	0.38	0.23
III	na	na	CBM	2	0.78	0.99	0.54
IV	1	0.05	PBM	0	0.02	0.02	0.05
V	1	0.05	PBM	1	0.24	0.22	0.32
VI	1	0.05	PBM	2	0.28	0.29	0.37
VII	1	0.15	PBM	0	0.08	0.02	0.08
VIII	1	0.15	PBM	1	0.19	0.23	0.17
IX	1	0.15	PBM	2	0.27	0.28	0.15
X	3	0.05	PBM	0	0.22	0.13	0.07
XI	3	0.05	PBM	1	0.18	0.15	0.08
XII	3	0.05	PBM	2	0.17	0.11	0.05
XIII	3	0.15	PBM	0	0.23	0.15	0.06
XIV	3	0.15	PBM	1	0.07	0.11	0.09
XV	3	0.15	PBM	2	0.09	0.09	0.06
XVI	5	0.05	PBM	0	0.26	0.18	0.11
XVII	5	0.05	PBM	1	0.24	0.17	0.07
XVIII	5	0.05	PBM	2	0.26	0.21	0.10
XIX	5	0.15	PBM	0	0.18	0.21	0.09
XX	5	0.15	PBM	1	0.22	0.17	0.14
XXI	5	0.15	PBM	2	0.19	0.11	0.08

Although well-defined priors for parameters may reduce such errors, these errors are still quite large compared with those for the proposed robust Bayesian method. Besides, it may be impossible to define priors accurately in many fisheries studies because of the limited knowledge about the studied fisheries. This makes the use of this amelioration method with informative priors difficult. We suggest the use of a robust Bayesian method such as one proposed in this study in assessing fisheries resources.

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References

Berger, J.O. 1985. Statistical decision theory and Bayesian analysis. Springer-Verlag, New York.

Box, G.E.P., and Tiao, G.C. 1992. Bayesian inference in statistical analysis. Wiley Classics Library Ed. John Wiley & Sons, New York.

Chen, Y. 1991. Growth comparison and analysis on thirty-three white sucker, *Catostomus commersoni*, populations in Ontario with reference to environmental variables. M.Sc. thesis, Zoology Department, University of Toronto, Toronto, Ont.

Chen, Y., and Andrew, N. 1998. Parameter estimation in modeling the dynamics of fish stock biomass: are currently used observation-error estimators reliable? Can. J. Fish. Aquat. Sci. **55**: 749–760.

Chen, Y., and Paloheimo, J.E. 1998. Can a more realistic model error structure improve parameter estimation in modeling the dynamics of fish populations? Fish. Res. **38**: 9–17.

Chen, Y., Jackson, D.A., and Paloheimo, J.E. 1994. Robust regression analysis of fisheries data. Can. J. Fish. Aquat. Sci. **51**: 1420–1429.

Cox, D.R., and Hinkley, D.V. 1974. Theoretical statistics. Chapman and Hall, London, U.K.

Dennis, B. 1996. Discussion: should ecologists become Bayesians? Ecol. Appl. **6**: 1095–1103.

Deriso, R.B., and Quinn, T.J., II. 1997. Quantitative fish dynamics. Oxford University Press, New York.

Deriso, R.B., Quinn, T.J., II, and Neal, P.R. 1985. Catch–age analysis with auxiliary information. Can. J. Fish. Aquat. Sci. **42**: 815–824.

Ellison, A.M. 1996. An introduction to Bayesian inference for ecological research and environmental decision-making. Ecol. Appl. **6**: 1036–1046.

Fournier, D.A. 1996. AUTODIF. A C++ array language extension with automatic differentiation for use in nonlinear modeling and statistic. Otter Research Ltd., Nanaimo, B.C.

Fournier, D.A., and Archibald, C. 1982. A general theory for analyzing catch at age data. Can. J. Fish. Aquat. Sci. **39**: 1195–1207.

Hilborn, R., and Walters, C.J. 1992. Quantitative fisheries stock assessment: choice, dynamics, and uncertainty. Chapman and Hall, New York.

Hilborn, R., Pikitch, E.K., and Francis, R.C. 1993. Current trends in including risk and uncertainty in stock assessment and harvest decisions. Can. J. Fish. Aquat. Sci. **50**: 874–880.

Kinas, P.G. 1996. Bayesian fishery stock assessment and decision making using adaptive importance sampling. Can. J. Fish. Aquat. Sci. **53**: 414–423.

Lawrence, K.D., and Arthur, J.L. 1990. Robust regression: analysis and applications. Marcel Dekker, Inc., New York.

Polacheck, T., Hilborn, R., and Punt, A.E. 1993. Fiting surplus production models: comparing methods and measuring uncertainty. Can. J. Fish. Aquat. Sci. **50**: 2597–2607.

Ricker, W.E. 1975. Computation and interpretation of biological statistics of fish populations. Bull. Fish. Res. Board Can. No. 191.

Rousseeuw, P.J., and Leroy, A.M. 1987. Robust regression and outlier detection. Wiley, New York.

Rubin, D.B. 1988. Using the SIR algorithm to simulate posterior distributions. *In* Bayesian statistics 3. *Edited by* J.M. Bernardo, M.H. de Groot, D.V. Lindley, and A.F.M. Smith. Clarendon Press, Oxford, U.K. pp. 395–402.

Schnute, J. 1989. The influence of statistical error on stock assessment: illustrations from Schaefer's model. *In* Effects of ocean variability on recruitment and an evaluation of parameters used in stock assessment models. *Edited by* R.J. Beamish and G.A. McFarlane. Can. Spec. Publ. Fish. Aquat. Sci. No. 108. pp. 101–109.

Smith, A.F.M., and Gelfand, A.E. 1992. Bayesian statistics without tears: a sampling–resampling perspective. Am. Stat. **46**: 84–88.

Taylor, B.L., Wade, P.R., Stehn, R.A., and Cochrane, J.F. 1996. A Bayesian approach for classification criteria for Spectacled Eiders. Ecol. Appl. **6**: 1077–1089.

Walters, C. 1998. Evaluation of quota management policies for developing fisheries. Can. J. Fish. Aquat. Sci. **55**: 2691–2705.

Walters, C.J., and Ludwig, D. 1994. Calculation of Bayes posterior probability distributions for key population parameters. Can. J. Fish. Aquat. Sci. **51**: 713–722.

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Cyclic dynamics of a yellow perch (*Perca flavescens*) population in an oligotrophic lake: evidence for the role of intraspecific interactions

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Abstract: Understanding the extent to which repeated oscillations in fish populations are driven by external factors or internal processes within the population is an important challenge. We document cyclic dynamics in a population of yellow perch (*Perca flavescens*) in oligotrophic Crystal Lake. Since 1981, we have observed three cases of cohort dominance in which two age-classes dominated the population for roughly 5 years. Young-of-the-year (YOY) perch were caught in 1981–1982, 1986–1987, and 1990–1991, whereas few to no YOY were caught during the midyears. The presence of YOY was negatively related to juvenile perch abundance and positively related to adult perch abundance. Mechanisms that may be responsible for these patterns include cannibalism of YOY by either juveniles or adults, potential for reproduction by adults, and competition between YOY and juveniles. YOY were abundant primarily in years when reproductively mature fish were in the lake, suggesting that the repeated oscillations are driven predominantly by pulses of abundant, reproductive, adult perch. As these young perch grow to juveniles, they exclude the possibility of survival by successive cohorts through cannibalistic and competitive interactions. This exclusion occurs until they themselves become reproductively mature and the cycle then repeats. Ultimately, long-term patterns in Crystal Lake suggest that cyclic dynamics are generated by intraspecific interactions.

Résumé : Établir dans quelle mesure les oscillations répétées des populations de poisson sont attribuables à des facteurs externes ou à des processus qui se déroulent à l'intérieur des populations constitue un défi important. Nous présentons des données sur la dynamique cyclique d'une population de perchaude (*Perca flavescens*) du lac Crystal, un lac oligotrophe. Depuis 1981, nous avons observé trois cas de dominance de cohorte dans lesquels deux classes d'âge ont dominé la population pendant environ 5 ans. Des jeunes de l'année ont été capturés en 1981–1982, 1986–1987 et 1990–1991, tandis que seulement quelques-uns ont été capturés, ou même aucun, entre ces années. La présence de jeunes de l'année était corrélée négativement avec l'abondance des perchaudes juvéniles, et corrélée positivement avec l'abondance des perchaudes adultes. Au nombre des facteurs possibles responsables de ces profils, on compte le cannibalisme exercé sur les jeunes de l'année par les juvéniles ou les adultes, le potentiel de reproduction des adultes et la compétition entre les jeunes de l'année et les juvéniles. Les jeunes de l'année étaient abondants surtout dans les années où des poissons sexuellement matures étaient présents dans le lac, ce qui laisse penser que les oscillations répétées sont principalement attribuables aux poussées d'abondance des perchaudes génitrices. Tandis que ces jeunes perchaudes croissent pour atteindre le stade juvénile, elles éliminent les possibilités de survie des cohortes suivantes par un comportement de cannibalisme et de compétition. Cette situation perdure jusqu'à ce que ces poissons atteignent eux-mêmes la maturité sexuelle; puis le cycle se répète. Ultimement, les profils de population à long terme dans le lac Crystal laissent penser que les dynamiques cycliques sont régies par des interactions intraspécifiques.

[Traduit par la Rédaction]

Introduction

Species population dynamics are controlled by a combination of external factors and processes that are generated internally in the population by the species itself. It is usually difficult to interpret this combination of causal factors due, in part, to interactions among the array of interacting organisms that occupy an ecosystem's food web (e.g., Maurer 1998). To better understand the fundamental ecology of organisms, it is advantageous to identify situations where population dynamics can be largely attributed to internally generated processes. Here, we report long-term fluctuations in a population of freshwater fish that are largely attributable to self-generated processes within a single habitat.

One of the most intriguing types of population fluctuation is that of regular cyclical change. Recurrent oscillations in the dynamics of natural populations have generated considerable and continuing ecological interest in both the occur-

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rence and cause of these cycles. Theoretical models of populations have shown that repeated oscillations can result from a number of factors including combinations of fertility and survival rates, density dependence, and predator–prey dynamics (Hassell et al. 1976; Nisbet and Gurney 1982). Cyclic patterns of abundance have been observed in freshwater fishes (e.g., Hamrin and Persson 1986), although the ability to detect such cycles is often obscured by the influence of environmental factors such as weather. While random fluctuations in fish populations are frequently attributed to variable recruitment success, the mechanisms driving cyclic fluctuations are less understood (Sissenwine et al. 1988). Because fishes are important resources for commercial and recreational users and because shifts in their populations can exert important influences over basic lake features, defining the processes driving population dynamics, whether cyclic or not, is of value to ecologists and managers alike. While processes determining population fluctuations across space and time may include both within-lake and external factors, many studies highlight the importance of internal processes such as predation, inter- and intra-specific competition, and cannibalism (see Sissenwine 1984; Post et al. 1998).

Perch are notorious for variable recruitment leading to strong year-classes or cohort dominance, as demonstrated in populations of European perch (*Perca fluviatilis*) (Menshutkin and Zhakov 1964) and yellow perch (*Perca flavescens*) (Forney 1971). Studies have attributed successful recruitment events to climate-related factors such as lake level fluctuation (Henderson 1985) and water temperature (Craig et al. 1979) and to food resources available during young-of-the-year (YOY) life development (Mills et al. 1989). The success of a cohort can be maintained through cannibalism (Alm 1952; Menshutkin and Zhakov 1964; Tarby 1974), competition within and between species (Alm 1946; Persson 1983; Henderson 1985), and predation (Forney 1971; Mills et al. 1987) and can result in domination by a particular age-class. The extent to which cohort dominance is a random event or may belong to a longer-term cyclic process is often unknown.

Long-term data sets provide a unique opportunity for distinguishing between "variable" and "repeated" events. A phenomenon such as cohort dominance that appears variable in the short term may instead be viewed as part of a longer-term pattern as the length of the time series grows (Magnuson 1990). In this study, we use a 16-year database to test for patterns of cyclic population fluctuation in a lake with a simple fish community, historically dominated by the yellow perch, for which preliminary observations suggested a clear pattern of cohort dominance. Our objectives are to describe the patterns in size structure of a yellow perch population through time, to explore potential mechanisms that might explain observed patterns including climate and food availability, and to consider the importance of reproduction, cannibalism, and competition in regulating dynamics in a population of yellow perch in a relatively uncomplicated system.

Methods

Study site

Crystal Lake is a small (35 ha), oligotrophic, sandy-bottom lake characterized by high water clarity and extremely low macrophyte abundance resulting in minimal habitat structure in the littoral zone. It has been studied since 1981 as part of the North Temperate Lakes Long Term Ecological Research (NTL LTER) program (Magnuson et al. 1997). As part of the LTER program, the fish community has been sampled each August since 1981 using a variety of gear types. Gear, sampling locations, and effort have been constant across time, allowing comparison between years (see Magnuson et al. 1994). Throughout most of these years, yellow perch have dominated the fish community, representing >95% of fish caught throughout the 1980s. More recently, yellow perch have been replaced as the dominant fish by the exotic rainbow smelt (*Osmerus mordax*) (Hrabik et al. 1998). Rainbow smelt, first discovered in Crystal Lake in 1985, became abundant starting in 1993, with maximum abundance recorded in 1996.

Yellow perch begin spawning when water temperatures reach roughly 8°C, with hatching occurring within about 8–20 days (Becker 1983). In Crystal Lake, spawning and hatching typically occur in the month of May.

Fish sampling

The abundance and size structure of the Crystal Lake fish community were measured annually. The littoral fish community was sampled at six sites using fyke nets, beach seines, and crayfish and minnow traps. Night beach seines were most effective at capturing smaller sized fishes (especially YOY), whereas fyke nets caught fish from a wide range of sizes. To assess fish in the pelagic zone, a set of seven vertically hung gill nets ranging in mesh size from 19 to 89 mm were set at the deepest point of the lake. Trammel nets set from the lake bottom and extending to the thermocline were used to sample fish in the thermocline region.

Fish caught were identified to species, measured, and a proportion of fish in each 10-mm size category weighed using a Pesola spring balance. Scales were also collected from the weighed yellow perch. Catch-per-unit-effort (CPUE) was calculated for each fish caught in each gear type by dividing the number of fish caught by both the number and the duration of the sets.

Abundance of adult yellow perch was estimated using a Simrad EY-M 70 khz echo sounder to perform night hydroacoustic surveys. Returning acoustic signals were recorded on both audiotape (1981–1987) and digital audiotape (1988–1994) and analyzed using the Hydroacoustic Data Aquisition software package to produce fish densities by size for discreet depths in the lake. Details of this protocol are described in Rudstam et al. (1993). Vertical distribution and species composition of fish caught in a suite of vertical gill nets placed near the transects were used to estimate the proportion of each species at each depth, allowing species composition to be assigned to the targets observed in the hydroacostic data set for each year. Acoustic data were used to assess fish greater than 75 mm; we were unable to distinguish species for fish smaller than this size, so no acoustic estimates are available for YOY fish. Further details regarding all of these fish sampling protocols can be found in Magnuson et al. (1994) and at the NTL LTER web site (http://limnology.wisc.edu).

Data generated from annual fish sampling include CPUE data for fish caught in sampling gear and abundance estimates derived from acoustic data. CPUE data are valuable for looking at shifts in population size structure across years and comparing the presence or absence of a given size category across years. Yet, because gear types are size selective, these data do not allow fine-scale quantitative comparison across size categories in a given year. Abundance estimates generated by hydroacoustics were better suited for addressing questions of general shifts in abundance of larger-sized fish because of greater differences in vertical distribution between adults of different species.

Yellow perch data analysis

To look for possible interactions between different size categories of perch, we grouped yellow perch into three categories designated as YOY, juvenile, and adult. Total length was used to

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distinguish categories. YOY were defined as a subset of the total number of fish less than 75 mm caught in beach seine hauls, as this was the only type of sampling gear in which we caught YOY fish. During years in which small fish were caught, it was important to determine whether individuals in these small size categories were YOY or 1+ fish. To do so, scale impressions for YOY and adult fish were made on acetate slides and read using a microfiche projector. Lengths of individuals confirmed to be YOY according to scale data were used to estimate a normal distribution of lengths in the YOY population in a given year. The maximum length of YOY in a given year was defined as two standard deviations from the mean. Among the beach seine fish, only those less than the defined maximum length in a given year were counted as YOY. YOY CPUE was calculated by dividing the number of YOY fish by the total number of sets, which was 18 in all but one year. We defined juvenile perch as those ranging in length from 75 to 135 mm and adult perch as those with lengths greater than 135 mm. Adult length was defined based on visual inspection of perch egg sacs collected in the fall of 1995. Inspection revealed that eggs from fish smaller than 135 mm were not fully developed. We therefore concluded that fish smaller than this size were incapable of reproduction.

Data, both CPUE and sonar abundance estimates, were summed for fish in these three size categories. We tested the comparability of acoustic and CPUE estimates for the juvenile and adult size categories using a correlation analysis for the years 1981–1995. CPUE and acoustic data were generally correlated for adult estimates ($r = 0.76$) and juvenile estimates ($r = 0.78$).

Fecundity and consumption estimates

Estimates of population fecundity and total consumption can suggest whether mechanisms such as reproduction, competition, and cannibalism might explain long-term patterns. We estimated population fecundity as the potential number of eggs spawned in the spring. To define the relationship between fecundity and adult perch total length, we used a dissecting microscope to count the number of eggs in egg sacs of female yellow perch captured in the late fall of 1995. We then fit a regression equation (eggs per female = 68.8 × length (millimetres) – 4030.0 ($r^2 = 0.55$)) and used it in conjunction with the August CPUE data from each year to derive an index of the number of eggs that could potentially have been produced by the perch population in the previous spring.

We estimated Crystal Lake yellow perch consumption in each year from 1981 to 1994 using the bioenergetics model developed by Kitchell et al. (1977). Our approach was to use estimates of thermal occupancy and size-at-age to estimate consumption from May through October (the growing season) in each year. To derive model input, we used a von Bertalanffy growth model (Ricker 1975) fit to scale data to estimate a length-at-age curve for Crystal Lake perch. We determined the proportion of the population in each age-class by applying minimum and maximum sizes for each age from the growth model to length–frequency data for all perch caught in each year; the total population estimates obtained from hydroacoustics data were then proportionated into a number of fish in each age-class. We determined the average temperature of the water occupied by perch in August of each year using vertical gillnet data and vertical temperature profiles. For the model, we grew perch at this occupied temperature. At times of the year when water temperatures in the lake were less than the occupied temperature, we grew perch at the highest temperature found in the lake. The total consumption for each age-class each year was then summed to obtain each yearly estimate of consumption for the total population.

Climate and zooplankton data

Ice breakup date was recorded annually. Water temperature and zooplankton abundances were measured every 2 weeks during the open-water season. Water temperature was measured at 1-m intervals throughout the water column. These values were then averaged to generate an average water column temperature for each date. Zooplankton samples were collected at 2-m intervals throughout the water column using a 2-m-long Schindler–Patalas trap, pooled across depths to generate a volume-weighted sample, counted, and biomasses estimated as detailed in Sanderson (1998). Biweekly temperature and zooplankton data for dates from June through August were averaged to generate yearly summer averages.

Statistical analysis

We used dynamic linear models (DLMs) to determine how the patterns observed for YOY related to climate, food availability, and juvenile and adult perch abundances. DLM are useful tools for detecting trends and determining explanatory variables in time series data (Pole et al. 1994; Cottingham and Carpenter 1998; Lamon et al. 1998). Unlike conventional statistical techniques (i.e., regression analysis, stock–recruit models), DLMs recognize that the sequencing of the data is critical such that the level of the response variable at a given time step may be strongly related to its level at one or more time steps earlier. These models further recognize that the passing of time diminishes the value of data for prediction and thereby places greater importance on data from recent time steps. Unlike other time series models, DLMs allow parameters to fluctuate through time to reflect shifts in both the dynamics of the system (i.e., the level of the response variable) and processes that drive these dynamics.

Each model was fit using two equations, an observation equation that relates parameters to the observed data and a system equation that defines the evolution of the parameters through time, as follows.

Observation equation:

$$Y_t = F_t' \, \theta_t + \nu_t, \quad \nu_t \sim N[0, V_t]$$

System equation:

$$\theta_t = G_t \theta_{t-1} + \omega_t, \quad \omega_t \sim N[0, W_t]$$

where Y_t is the observation at time t, YOY abundance in this case, F_t' is the regression vector of predictors, θ_t is a vector of the model parameters that, depending on the model, includes level, growth, and regressor parameters, ν_t is a stochastic error term having a normal distribution with zero mean and observation variance V_t, G_t is a matrix of known coefficients that defines the systematic evolution of the parameters across time, and ω_t is a stochastic error term having a normal distribution with a mean of zero and covariance matrix W_t. This latter covariance matrix is where weighting of observations depending on age occurs, as covariances are in part defined using the discount factor (Pole et al. 1994).

The discount factor indicates the extent to which the model considers prior observations when making predictions and can be altered to emphasize the importance of recent observations when making predictions about the next time step. The discount factor can vary from 0 to 1, although discount factors lower than 0.8 are rarely used in fits of ecological time series (Pole et al. 1994). A value of 1 means that observations are not discounted at all, and each observation is considered equal, independent of how long ago it was observed. A discount factor of 0 means that the model does not include any old information and the regression depends entirely on new observations. Generally, the lower the discount factor the more rapidly the model adapts to changes in the time series.

The analysis was composed of a two-step process in which we first fit a forward-looking model, referred to as the "on-line" model, and then using the information learned from this model, we fit a retrospective or backward-looking model. We used a Bayesian learning process to fit the on-line model. This is a multistep, iterative process that involves combining prior beliefs about a response

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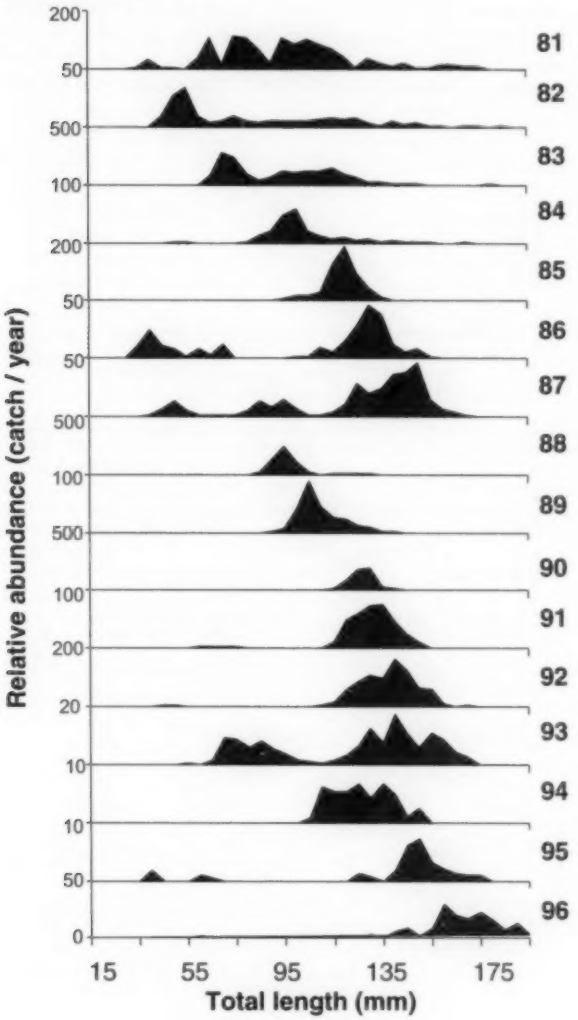
Fig. 1. Relative abundance (CPUE) of yellow perch in Crystal Lake as a function of size for the years 1981–1996. Data in each year include fish caught from multiple gear types set at different habitats within the lake.



variable with information about the probability of observing that response (likelihood) to generate a prediction for the next time step, observing the actual event, comparing the prediction with the true observation, and then generating a modified view of that prediction given what we have just learned. The modified view becomes the prediction for the next time step after the addition of variance to account for the passage of time (discounting) and the process repeats. Because our goal was to explain an observed pattern and understand what had already happened rather than formulate predictions, we used a retrospective analysis, also referred to as "smoothing analysis" (West and Harrison 1989). This analysis allows us to use the information learned in the on-line analysis to hindcast. This results in both a smoothing effect and an increased confidence in the predictions given that we were able to use information from all observations in the time series to hindcast each point. The analysis was conducted using the BATS statistical package (Pole et. al 1994). Details of the modeling process are described in West and Harrison (1989), Pole et al. (1994), Cottingham and Carpenter (1998), and Lamon et al. (1998, 1999).

We began with the simplest possible model that consisted of a "random walk" about the mean with no systematic growth trend or regressor variables. Subsequent models were evaluated relative to the initial random walk model by calculating the difference in log likelihoods for each model. Log likelihood is a measure of the likelihood of the model given the data (Pole et. al 1994). Better models have larger likelihoods, as they indicate a greater likelihood of one model relative to another. Evaluating models using the difference in log likelihood is an equivalent expression of the likelihood ratio (Lamon et al. 1998). A difference of 1 (–1) represents "evidence for" (against) a model and the confidence in the model increases as this difference increases such that a difference of 10 (–10) indicates strong evidence for (against) a model (Lamon et al. 1999).

A variety of models were fit with the goal of identifying possible explanatory variables and from these determining which model was best at explaining the dynamics in YOY yellow perch. Specific variables considered included May and summer water temperatures, annual ice thaw date, May and summer *Daphnia* and zooplankton biomasses, and juvenile and adult perch abundances. All data were standardized before analysis. Temperatures and zooplankton biomasses from the month of May were considered because they measure conditions when spawning and hatching of YOY occur. Similarly, summer values indicate conditions that could have impacted YOY during their first summer.

Results

The size structure of Crystal Lake's yellow perch population for fish caught in all gear types differed strongly among years (Fig. 1). YOY were present in some years and absent in others. Cohort dominance was seen three different times throughout the record. YOY classes that succeeded in growing to adulthood were present in pairs of years including 1981 and 1982, 1986 and 1987, and 1991 and 1992. Fewer individual YOY were caught in 1991 and 1992, making these cohorts less evident in Fig. 1, although the peak associated with these cohorts is apparent starting in 1993. Few to no YOY were caught in the 2–3 years following successful recruitment events (i.e., 1988, 1989, 1990). The year 1983 is a slight exception; YOY CPUE in this year was more than an order of magnitude lower than that observed in 1981 and 1982 but still large enough to be quantified as a "high-YOY" year relative to other years. The year-class from 1983, however, did not result in cohort dominance. Finally, adult populations observed in the latter year of each couplet were absent in the following year (i.e., 1983, 1988) with the exception of 1993. The die-off of adult perch may result from a combination of spawning stress and overwinter mortality (Wooton 1979; Hayes and Taylor 1990) and may be exacerbated by competition between YOY and adult perch for limited zooplankton resources that they share (Hamrin and Persson 1986; Persson 1987). Zooplankton biomass tends to be lower in years with large YOY cohorts, suggesting that a reduction in zooplankton resources by abundant YOY negatively impacts adult survival (Sanderson 1998). The die-off of adult perch does not result from harvesting, as there is no commercial fishing and recreational harvest is minimal owing to the small size of the perch.

These oscillating patterns in abundance again emerged when data were aggregated into the YOY, juvenile, and adult categories. In general, YOY were found in years when adult

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Fig. 2. Abundance of (*a*) YOY, (*b*) juvenile, and (*c*) adult yellow perch. YOY data are CPUE estimates, and juvenile and adult abundances were estimated using acoustic data. Years in which YOY were abundant and continued to dominate the population over the following 3–4 years are underlined and represented by open circles.

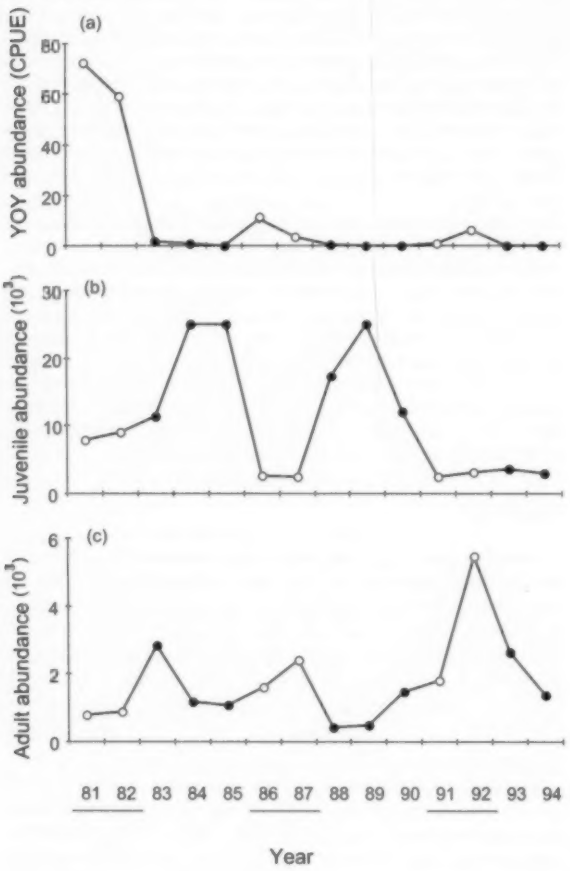
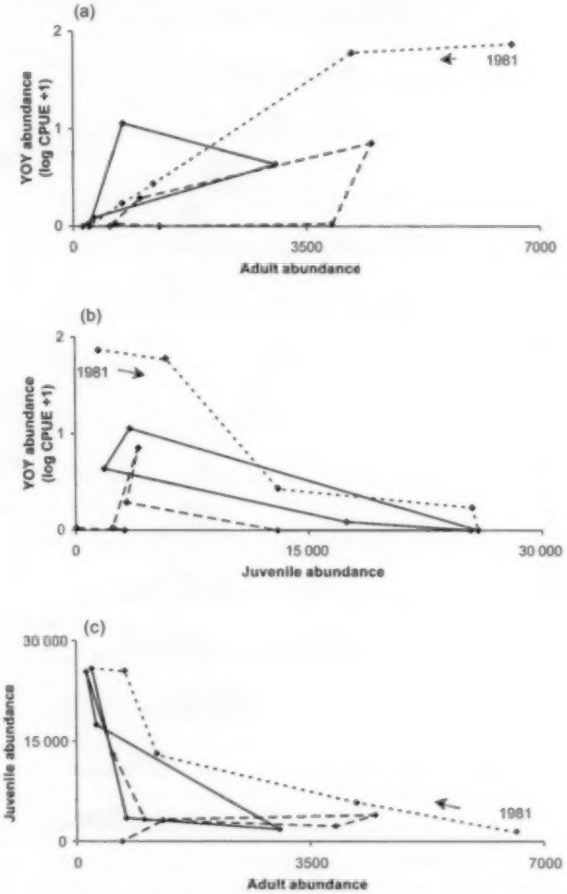


Fig. 3. Phase diagrams plotting abundances of (*a*) YOY versus adult yellow perch, (*b*) YOY versus juvenile yellow perch, and (*c*) juvenile versus adult yellow perch. Each line type represents a different 5-year period of the time series (short-dashed line, 1981–1985; solid line 1986–1990; long-dashed line, 1991–1995). The starting point is indicated by "1981" and the arrow in each panel. YOY data are log(CPUE + 1), and juvenile and adult population estimates are derived from acoustic data.



abundance was high and juvenile abundance was low (Fig. 2). In contrast, YOY were not observed in years when juvenile abundance was extremely high. Phase plots of one age category (i.e., YOY) versus another (i.e., juvenile) illustrate both the direction of the relationships and the cyclic nature of the dynamics (Fig. 3). Each 5-year period in the time series is indicated by a different line style to highlight the cyclic nature of these dynamics. YOY and adult abundance estimates move in positive sloping cycles, with years of extremely low adult abundances coinciding with years with no YOY (Fig. 3*a*). In contrast, YOY and juvenile abundances oscillate in a negative direction, as do adult and juvenile abundances (Figs. 3*b* and 3*c*). In both of these latter cases, high abundance of one group is associated with low abundance of the other, again suggesting the negative nature of interactions between these groups.

Results from DLMs fit to explain YOY abundance across years indicate that water temperature and ice thaw date were unable to explain the dynamics in YOY abundance, as indicated by the more negative log likelihoods of these models relative to the random walk model (Table 1). There was some evidence that YOY abundance was related to May *Daphnia* abundance. Overall, the best-fit model was one having linear growth (i.e., the "level" of YOY abundance was systematically changing over time) and regressor terms for both juvenile and adult perch, as indicated by a difference in model log likelihoods of 6.6 (Table 1). The fit also improved when the trend discount was lowered to 0.8. A discount factor of 0.8 means that the model uses the previous two to four observations when estimating parameters and in this case implies that YOY abundance in a given year is most strongly influenced by the conditions defined in the 2–4 years prior.

YOY abundance across years was successfully explained using a model containing terms for linear growth and juvenile and adult CPUE. The retrospective level of YOY abundance is represented by the line and 90% credible intervals

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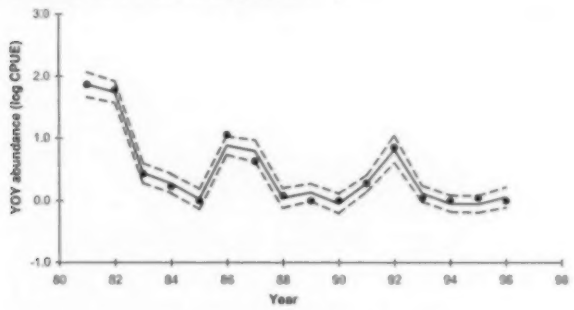
Table 1. Results of DLM fitting to YOY yellow perch abundance data in Crystal Lake.

Model parameter	Trend discount	Mean square error	Mean absolute deviation	log likelihood	Difference in log likelihood from random walk model
Random walk	0.9	0.406	0.520	−19.0	
Linear growth	0.9	0.469	0.499	−18.0	1
Water temperature*					
May	0.9	1.60	0.859	−28.0	−9.0
Summer	0.9	1.32	0.825	−25.1	−6.1
Ice breakup date*	0.9	0.459	0.524	−35.6	−16.6
Zooplankton biomass*					
May *Daphnia*	0.9	0.48	0.54	−14.7	4.3
May total	0.9	1.01	0.69	−19.8	−0.8
Summer *Daphnia*	0.9	1.62	1.62	−27.4	−8.4
Summer total	0.9	0.59	0.56	−23.3	−4.3
Yellow perch*					
Juvenile	0.9	0.354	0.429	−19.1	−0.1
Adult	0.9	0.432	0.420	−19.8	−0.8
Juvenile, adult	0.9	0.546	0.480	−14.6	4.4
	0.8	0.436	0.439	−12.4	6.6

Note: The random walk model presented in the first row served as the basis for evaluating subsequent models. Included are the trend discount factor used to fit each model, model mean square error, maximum absolute deviation, and log likelihood. Differences in log likelihoods between the random walk and subsequent models are presented in the final column. The more positive this number, the stronger the evidence for a particular model. Parameters considered include a linear growth parameter and the regression variables average May and summer water temperatures (°C), annual date of ice thaw, average May and summer zooplankton biomasses (*Daphnia* and total zooplankton biomass, µg/L), and CPUE estimates of adult and juvenile yellow perch.

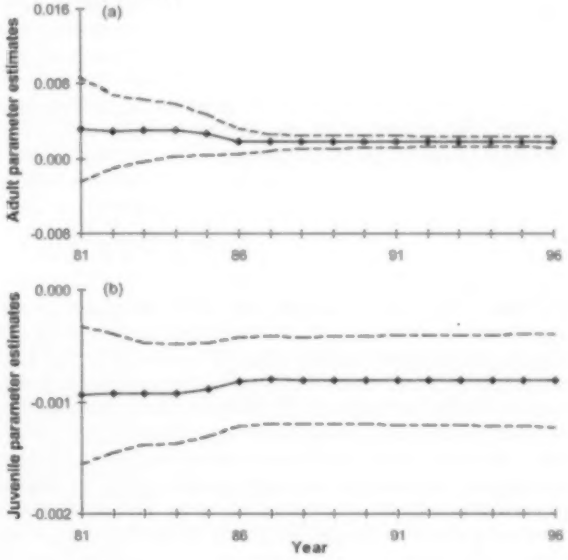
*Models including regressor variables were fit using linear growth.

Fig. 4. Retrospectively fit model predictions (solid line), 90% confidence bands (dashed lines), and actual observations (circles) of YOY yellow perch abundance from 1981 to 1996. YOY abundance is shown as log(CPUE + 1).



in Fig. 4. The model successfully captures the dynamics in YOY abundance. Observed data (circles) fell within the 90% probability intervals in all but one case. The parameter coefficients that we have estimated here suggest both the direction and strength of correlation between regressor variables and YOY abundance, although a slight correlation between these two regressor variables (r = 0.04) means that interpretations must be viewed with some degree of caution. Coefficients for the juvenile parameter were negative throughout the entire time series (Fig. 5*b*), whereas coefficients for the adult parameter were positive (Fig. 5*a*). Furthermore, the absolute value of the adult coefficients was consistently greater than the juvenile coefficients. In both cases, the absolute value of the coefficient was greater early in the time series and leveled within about 6 years, or about one cycle length into the time series.

Fig. 5. (*a*) Adult and (*b*) juvenile yellow perch parameter estimates for the retrospective model fit to explain YOY abundance for each year of the time series. Note that juvenile parameter estimates are negative, whereas adult parameter estimates are positive.



Discussion

The pattern of perch abundance observed in Crystal Lake appears cyclic and associated with successful reproduction

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Table 2. Categorization of YOY yellow perch abundance, population fecundity index, and population consumption estimates into groups.

Year	YOY	Population fecundity index	Consumption
1981	High	High	Low
1982	High	Moderate	Low
1983*	High	Moderate	High
1984	Low	Low	High
1985	Low	Low	High
1986	High	Low	Low
1987	High	High	Low
1988	Low	Low	High
1989	Low	Low	High
1990	Low	Low	High
1991	High	Moderate	Low
1992**	High	High	Low
1993**	Low	Moderate	Low
1994**	Low	Low	Low
1995**	Low	Moderate	na
1996**	Low	High	na

Note: YOY were divided according to number of fish caught (high, >15; low, <15), population fecundity according to the number of eggs in the population (high, >300 000; moderate, 100 000 – 300 000; low, <100 000), and consumption by juvenile and adult perch into high (>600 000 g/season) and low (<600 000 g/season) years. High-YOY years (underlined) tend to correspond to high to moderate fecundity and low consumption, whereas low-YOY years typically correspond to low fecundity and high consumption. na, not available.

*YOY in 1983 did not result in cohort dominance as in other years.

**Years when rainbow smelt were abundant in the lake.

occurring at roughly 5-year intervals (see Table 2). The long-term data and subsequent model results suggest that a number of potential mechanisms may be driving this repeated pattern of cohort dominance, but these mechanisms all appear to be generated from within the population. None of the climate variables were related to the dynamics in YOY. We believe that the positive relationship of YOY and May *Daphnia* biomass may be an artifact of lower juvenile and adult abundances and subsequent lower predation rates on zooplankton. This positive relationship is unlikely related to a direct interaction between YOY and *Daphnia*, as gape restrictions limit the ability of newly hatched YOY perch to ingest *Daphnia* early in the summer (Schael et al. 1991; T. Hrabik, 680 N. Park Street, Madison, WI 53706, U.S.A., unpublished data). Both juvenile and adult abundances were significant in explaining YOY abundance across years, although the direction of their impacts differs. The negative relationship between juvenile abundance and YOY perch contrasts with the positive relationship shown with adults. Mechanisms that might explain the negative impact of juveniles on YOY include cannibalism and competition, whereas the positive relationship with adult perch is likely a result of reproductive output and (or) a reduction in the abundance of juvenile perch. It is impossible to fully differentiate between these explanations given the cyclic dynamics and subsequent correlations between YOY, juvenile, and adult groups.

Adult–YOY interactions

The presence of YOY in the lake is clearly dependent on whether there are reproductively mature adults in the population. In a number of years (i.e., 1984, 1985, 1988, 1989), few to no fish greater than 135 mm were caught, suggesting that little to no reproduction could have occurred in that year. This observation is reflected in our estimates of reproductive potential defined as the number of eggs in the female population (Table 2). Total egg production was lowest in years when few to no YOY were caught in the lake. In contrast, high egg production coincided with successful YOY year-classes (i.e., 1981, 1987, 1992). Other studies of perch populations have reported an inverse relationship between recruitment and parental stock size, implying the importance of density-dependent interactions (LeCren et al. 1977). Similarly, studies that highlight cannibalism as a mechanism of population regulation indicate that recruitment occurs only after the adult population has largely disappeared (Alm 1952; Menshutkin and Zhakov 1964). In our study, however, the data indicate a strong positive relationship between adults and YOY, with recruitment occurring after numerically dominant juvenile populations have diminished in abundance. Furthermore, the magnitudes of the parameter coefficients in the final model indicate that adults are more important in explaining YOY abundance than are juveniles. Cannibalism by non-YOY perch does occur at a low rate (Hrabik et al. 1998); however, the positive reproductive role of adults clearly outweighs any negative cannibalistic impact. From this, we deduce that variation in reproduction is the primary mechanism explaining variation in YOY abundance across years, and competition and cannibalism act within that context to maintain the cyclic pattern.

Juvenile–YOY interactions

The negative relationship between juveniles and YOY suggests the potential for competitive and (or) cannibalistic interactions. Intraspecific competition can result in limited size diversity in fish populations, a pattern that is often intensified in structurally simplified habitats such as Crystal Lake (Persson 1983; Diehl 1993). Persson (1983) suggested that competitive interactions between two consecutive age-classes could result in reduced growth rates of the younger age-class. YOY survival is highly dependent on growth in the early developmental stages (Houde 1989) such that reduced growth rates resulting from competition with older year-classes could end in either death by starvation or increased vulnerability to predation. The stunted nature of Crystal Lake's perch population suggests that food resources are generally limiting and competition between size-classes likely (see Alm 1946; Hayes and Taylor 1990). Moreover, diets of adult and juvenile perch in Crystal Lake are dominated by zooplankton (Hrabik et al. 1998), which are also the dominant food resource for YOY perch throughout most of the summer season (Schael et al. 1991; Post and McQueen 1994). Thus, it is reasonable to infer that any reduction in growth owing to competition of an already food-limited population could strongly affect YOY survival and subsequently eliminate that entire age-class.

This inference contrasts with other perch studies that have shown that younger age-classes are competitively superior because they have lower metabolic costs and greater prey capture rates than older fish (Hamrin and Persson 1986; Persson 1987). We cannot assess the nature of competitive interactions between YOY and juveniles given the data at hand. However, if YOY in Crystal Lake were competitively

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superior to juvenile perch, as Harmin and Persson (1986) and Persson (1987) might suggest, their absence in some years more likely results from cannibalism by juveniles.

Cannibalistic interactions might explain the inverse relationship observed between juveniles and YOY. Piscivorous yellow perch can typically consume prey up to 40% of their own body length (Knight et al. 1984). YOY perch in Crystal Lake are typically 40–55 mm in early August, suggesting that they are vulnerable to cannibalism by juvenile perch (75–135 mm) for much of the summer season. Furthermore, perch diets analyzed in Crystal Lake indicate that some level of cannibalism by perch spanning both juvenile and adult size categories does occur (Hrabik et al. 1998). Although these data do not permit us to quantify cannibalism of YOY perch, evidence from other systems indicates that mortality of YOY due to cannibalism can be significant (Alm 1952; Tarby 1974; Post and Evans 1989). Furthermore, juvenile fish tend to have a greater cannibalistic impact because of their numerical dominance (Post et al. 1998), a pattern that is also likely in Crystal Lake where the abundance of juvenile yellow perch is far greater than that of adult yellow perch.

Our evidence suggests that cannibalism by juvenile fish may in part explain the inverse relationship between juvenile and YOY abundance. More interesting, however, is the notion that it contributes to the long-term cyclic dynamics of the Crystal Lake perch population. Periodic fluctuations in perch populations have been attributed to cannibalism by adults (Alm 1946; Menshutkin and Zhakov 1964). In these studies, cannibalism by adult perch on smaller perch prevented successful recruitment of YOY until the adult population was substantially reduced, thereby releasing YOY from predation constraints. Post and Evans (1989) speculated that slower growth rates resulting from intense competition with juveniles may intensify cannibalism and subsequently curb variation in year-to-year survival of YOY. While these cited studies focussed on adults as cannibals, our data indicate that perch in juvenile size categories may equally prey upon YOY, ultimately having the same regulatory impact as other studies have reported for adult perch. Thus, the positive effect of adult perch may, in part, result from a reduction in both the numbers of juveniles and the effect that juveniles may have on YOY through cannibalism and competition.

Our data cannot specifically address the relative importance of competition or cannibalism between YOY and juvenile perch, yet they do suggest that both mechanisms are likely influencing perch dynamics. Consumption by juvenile perch, whether competitive or cannibalistic in nature, would negatively impact YOY perch. This observation was supported by our estimates of consumption that were greatest in years with no YOY (Table 2). Furthermore, in years with unsuccessful recruitment, these two processes may interact such that competitive interactions result in slower growth of YOY, thereby prolonging their vulnerability to predation, a conclusion supported by a number of other studies (see Olson et al. 1995; Post et al. 1998).

Summary

Several studies have established the importance of intra-specific interactions in regulating perch population dynamics (Menshutkin and Zhakov 1964; Hamrin and Persson 1986; Post and Evans 1989). Many of these studies have been short-term attempts to evaluate specific mechanisms, yet few have been able to place the observed dynamics in a long-term context. Our 16-year record allowed us to observe three cycles of cohort dominance. Although the cycles observed here do not mimic the uniform periodicity generated from theoretically modeled populations, perfect cycles are unexpected in natural populations, largely because of the influence of fluctuating environmental factors.

Crystal Lake is in many ways a model system for addressing questions regarding cycles in the dynamics of fish populations because it is a small lake and its food web and habitat structure are relatively simple. The patterns observed in Crystal Lake support the notion that periodicity is more likely in systems with simple food chains (Pielou 1974). The fish community in Crystal Lake was essentially a single-species system throughout the 1980s such that dynamics were entirely driven by interactions within the perch population. Similar patterns have been reported in other systems having fish communities dominated by individual species (Menshutkin and Zhakov 1964; Post et al. 1998). The invasion and increase in rainbow smelt abundance in the early 1990s in Crystal Lake provide an opportunity to evaluate whether the cyclic dynamics can be maintained in a two-species system. While there is evidence that Crystal Lake's yellow perch abundance and growth rates have declined since the increase in rainbow smelt, it is unclear whether these changes will shorten, lengthen, or eliminate the periodic fluctuations in the perch population.

The periodicity in perch population dynamics has implications for other ecological features of Crystal Lake and provides an excellent example of the linkages between species and ecosystems. Shifts in the size structure of the perch population have implications for the consumption and cycling of nutrients by other trophic levels and ultimately impact other system features such as water clarity (Sanderson 1998). Such links between ecosystem properties and periodic behavior in a population can only be verified through the coordinated collection of diverse long-term data.

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References

Alm, G. 1946. Reasons for the occurrence of stunted fish populations with special reference to perch. Inst. Freshwater Res. Drottningholm Rep. **25**.

Alm, G. 1952. Year class fluctuations and span of life of perch. Inst. Freshwater Res. Drottningholm Rep. **33**: 17–38.

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Becker, G.C. 1983. Fishes of Wisconsin. University of Wisconsin Press, Madison, Wis.

Cottingham, K.L., and Carpenter, S.R. 1998. Population, community, and ecosystem variates as ecological indicators: phytoplankton responses to whole-lake enrichment. Ecol. Appl. **8**: 508–530.

Craig, J.F., Kipling, C., LeCren, E.D., and McCormack, J.C. 1979. Estimates of the numbers, biomass and year-class strengths of perch (*Perca fluviatilis* L.) in Windermere from 1967 to 1977 and some comparisons with earlier years. J. Anim. Ecol. **48**: 315–325.

Diehl, S. 1993. Effects of habitat structure on resource availability, diet and growth of benthivorous perch, *Perca fluviatilis*. Oikos, **67**: 403–414.

Forney, J. L. 1971. Development of dominant year classes in a yellow perch population. Trans. Am. Fish. Soc. **100**: 739–749.

Hamrin, S.F., and Persson, L. 1986. Asymmetrical competition between age classes as a factor causing population oscillations in an obligate planktivorous fish species. Oikos, **47**: 223–232.

Hassell, M.P., Lawton, J.H., and May, R.M. 1976. Patterns of dynamical behaviour in single-species populations. J. Anim. Ecol. **45**: 471–486.

Hayes, D.B., and Taylor, W.B. 1990. Reproductive strategy in yellow perch (*Perca flavescens*): effects of diet ontogeny, mortality, and survival costs. Can. J. Fish. Aquat. Sci. **47**: 921–927.

Henderson, B.A. 1985. Factors affecting growth and recruitment of yellow perch, *Perca flavescens* Mitchill, in South Bay, Lake Huron. J. Fish Biol. **26**: 449–458.

Houde, E.D. 1989. Subtleties and episodes in the early life of fishes. J. Fish Biol. **35**: 29–48.

Hrabik, T.R., Magnuson, J.J., and McLain, A.S. 1998. Predicting the effects of rainbow smelt on native fishes in small lakes: evidence from long-term research on two lakes. Can. J. Fish. Aquat. Sci. **55**: 1364–1371.

Kitchell, J.F., Stewart, D.J., and Weininger, D. 1977. Applications of a bioenergetics model to yellow perch (*Perca flavescens*) and walleye (*Stizostedion vitreum vitreum*). J. Fish. Res. Board Can. **34**: 1922–35.

Knight, R.L., Margraf, F.J., and Carline, R.F. 1984. Piscivory by walleye and yellow perch in western Lake Erie. Trans. Am. Fish. Soc. **113**: 677–693.

Lamon, E.C., Carpenter, S.R., and Stow, C.A. 1998. Forecasting PCP concentrations in Lake Michigan salmonids: a dynamic linear model approach. Ecol. Appl. **8**: 659–668.

Lamon, E.C., III, Carpenter, S.R., and Stow, C.A. 1999. Rates of decrease of polychlorinated biphenyl concentrations in five species of Lake Michigan salmonids. Can. J. Fish. Aquat. Sci. **56**: 53–59.

LeCren, E.D., Kipling, C., and McCormack, J.C. 1977. A study on the numbers, biomass and year-class strengths of perch (*Perca fluviatilis* L.) in Windermere from 1941 to 1966. J. Anim. Ecol. **46**: 281–307.

Magnuson, J.J. 1990. Long-term ecological research and the invisible present. BioScience, **40**: 495–501.

Magnuson, J.J., Benson, B.J., and McLain, A.S. 1994. Insights on species richness and turnover from long-term ecological research: fishes in north temperate lakes. Am. Zool. **34**: 437–451.

Magnuson, J.J., Kratz, T.K., Allen, T.F., Armstrong, D.E., Benson, B.J., Bowser, C.J., Bolgrien, D.W., Carpenter, S.R., Frost, T.M., Gower, S.T., Lillesand, T.M., Pike, J.A., and Turner, M.G. 1997. Regionalization of long-term ecological research (LTER) on north temperate lakes. Verh. Int. Ver. Limnol. **26**: 522–528.

Maurer, B.A. 1998. Ecological science and statistical paradigms: at the threshold. Science (Washington, D.C.), **279**: 502–503.

Menshutkin, V.V., and Zhakov, L.A. 1964. Mathematical determination of the nature of the population dynamics of perch in given ecological conditions. Ozera Karel'skogo Peresheika Limnologiya i Metodika Issledovanii. pp. 140–154.

Mills, E.L., Forney, J.L., and Wagner, K.J. 1987. Fish predation and its effect on the Oneida Lake food chain. *In* Predation: direct and indirect impacts on aquatic communities. *Edited by* W.C. Kerfoot and A. Sih. University Press of New England, Hanover, N.H. pp. 118–131.

Mills, E.L., Sherman, R., and Robson, D.S. 1989. Effect of zooplankton abundance and body size on growth of age-0 perch (*Perca flavescens*) in Oneida Lake, New York, 1975–86. Can. J. Fish. Aquat. Sci. **46**: 880–886.

Nisbet, R.M., and Gurney, W.S.C. 1982. Modelling fluctuating populations. Wiley, New York.

Olson, M., Mittelbach,G.G., and Osenberg, C.W. 1995. Competition between predator and prey: resource-based mechanisms and implications for stage-structured dynamics. Ecology, **76**: 1758–1771.

Persson, L. 1983. Food consumption and competition between age classes in a perch *Perca fluviatilis* population in a shallow eutrophic lake. Oikos, **40**: 197–207.

Persson, L. 1987. The effects of resource availability and distribution on size class interactions in perch, *Perca fluviatilis*. Oikos, **48**: 148–160.

Pielou, E.C. 1974. Population and community ecology: principles and methods. Gordon and Breach Science Publishers, New York.

Pole, A., West, M., and Harrison, J. 1994. Applied Bayesian forecasting and time series analysis. Chapman and Hall, New York.

Post, D.M., Kitchell, J.F., and Hodgson, J.R. 1998. Interactions among adult demography, spawning date, growth rate, predation, overwinter mortality, and the recruitment of largemouth bass in a northern lake. Can. J. Fish. Aquat. Sci. **55**: 2588–2600.

Post, J.R., and Evans, D.O. 1989. Experimental evidence of size-dependent mortality in juvenile yellow perch. Can. J. Zool. **67**: 521–523.

Post, J.R., and McQueen, D.J. 1994. Variability in first-year growth of yellow perch (*Perca flavescens*): predictions from a simple model, observations, and an experiment. Can. J. Fish. Aquat. Sci. **51**: 2501–2512.

Ricker, W.E. 1975. Computation and interpretation of biological statistics of fish populations. Bull. Fish. Res. Board Can. No. 191.

Rudstam, L.G., Lathrop, R.C., and Carpenter, S.R. 1993. The rise and fall of a dominant planktivore: direct and indirect effects on zooplankton. Ecology, **74**: 303–319.

Sanderson, B.L. 1998. Factors regulating water clarity in northern Wisconsin lakes. Ph.D. thesis, University of Wisconsin–Madison, Madison, Wis.

Schael, D.M., Rudstam, L.G., and Post, J.R. 1991. Gape limitation and prey selection in larval yellow perch (*Perca flavescens*), freshwater drum (*Aplodinotus grunniens*), and black crappie (*Pomoxis nigromaculatus*). Can. J. Fish. Aquat. Sci. **48**: 1919–1925.

Sissenwine, M.P. 1984. Why do fish populations vary? *In* Exploitation of marine communities. *Edited by* R.M. May. Springer-Verlag, New York. pp. 59–94.

Sissenwine, M.P., Fogarty, M.J., and Overholtz, W.J. 1988. Some fisheries management implications of recruitment variability. *In* Fish population dynamics. *Edited by* J.A. Gulland. John Wiley & Sons, Inc., New York. pp. 129–152.

Tarby, M.J. 1974. Characteristics of yellow perch cannibalism in Oneida Lake and the relation to the first year of survival. Trans. Am. Fish. Soc. **103**: 462–471.

West, M., and Harrison, P.J. 1989. Bayesian forecasting and dynamic models. Springer-Verlag, New York.

Wooton, R.J. 1979. Energy costs of egg production and environmental determinants of fecundity in teleost fishes. Symp. Zool. Soc. Lond. **44**: 133–159.

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Nocturnal habitat use of Atlantic salmon parr in winter

Kevin G. Whalen and Donna L. Parrish

Abstract: We completed 22 night snorkeling surveys between November and March 1995–1997 to quantify Atlantic salmon (*Salmo salar*) parr habitat use relative to habitat availability in the Rock River, Vermont, U.S.A. On average, post-young-of-the-year (PYOY) parr selected greater water depths in winter than young-of-the-year (YOY) parr, whereas YOY and PYOY parr both selected water velocities (≤19 cm/s) that were significantly lower than random measurements (46 cm/s). Maturity of PYOY parr had no significant influence on habitat selection. The majority of YOY and PYOY parr at night were found in contact with the stream bottom resting on silt–sand or gravel substrates in velocity dead-zone habitats created by the stream edge or depositional habitats created by midstream rocks and boulders. The strong selection that nocturnal Atlantic salmon parr exhibit for low water velocity areas in winter indicates the importance of maintaining large instream cover that provides refuges from high flows. The similarity that YOY and PYOY parr exhibited in many elements of habitat selection suggests that both stages may be similarly susceptible to habitat limitations in winter.

Résumé : Nous avons réalisé 22 relevés nocturnes en plongée libre de novembre à mars de 1995 à 1997 pour quantifier l'utilisation par des tacons de saumon atlantique (*Salmo salar*) de leur habitat en rapport avec la disponibilité de l'habitat dans la rivière Rock, au Vermont (É.-U.). En moyenne, les tacons de plus de 1 an se tenaient en hiver dans des eaux plus profondes que les jeunes tacons de l'année, tandis que les deux groupes se tenaient dans des eaux dont la vitesse (≤19 cm/s) était significativement inférieure à celle indiquée par nos mesures aléatoires (46 cm/s). La maturité des tacons de plus de 1 an n'influait pas de façon significative sur le choix de l'habitat. La majorité des jeunes tacons de l'année et des tacons de plus de 1 an reposaient la nuit sur le fond du cours d'eau, sur des substrats de limon et de sable ou de gravier et en eaux stagnantes en bordure du cours d'eau ou dans des zones de dépôt créées par des pierres et des blocs rocheux présents au milieu du cours d'eau. La préférence nocturne marquée des tacons de saumon atlantique pour les zones à faible courant en hiver indique qu'il est important de maintenir dans les cours d'eau un couvert important offrant des abris contre les forts débits. Comme les jeunes tacons de l'année et les tacons de plus de 1 an ont bien des points en commun quant au choix de leur habitat, on peut penser qu'ils pourraient être affectés de façon similaire par les limitations hivernales en matière d'habitat.

[Traduit par la Rédaction]

Introduction

Studies of winter habitat selection of salmonids have identified critical habitats (Cunjak 1988; Griffith and Smith 1993; Heggenes et al. 1993; Brown and MacKay 1995) and mechanisms by which conditions in winter may affect changes in population density (Tschaplinski and Hartman 1983). A number of factors must be integrated in winter habitat selection as environmental conditions change. In winter, environmental conditions may impose significant constraints on fish activity; habitats may experience rapid alteration from ice formation (Power et al. 1993; Whalen et al. 1999), low water temperatures may affect motor capabilities (Rimmer et al. 1985), and extended cold periods may necessitate using stored energy for the fulfillment of metabolic needs (Cunjak and Power 1986*a*). Environmental conditions affecting parr activity and habitat requirements and thus winter survival may vary temporally (Cunjak and Randall 1993; Power et al. 1993) and spatially (Berg 1994; Brown and MacKay 1995). For Atlantic salmon (*Salmo salar*) parr, winter mortality has been estimated to be as high as 60–70% (Cunjak and Randall 1993; Orciari et al. 1994). Because high winter mortality can impact smolt production, understanding the relationship between parr habitat selection and habitat availability, across both temporal and spatial scales, is necessary to evaluate the causes of winter mortality and its effect on smolt recruitment.

Fish age and size may affect activity patterns and in turn winter habitat selection (Cunjak and Power 1986*b*; Heggenes et al. 1993). For Atlantic salmon, young-of-the-year (YOY) and post-young-of-the-year (PYOY) parr may

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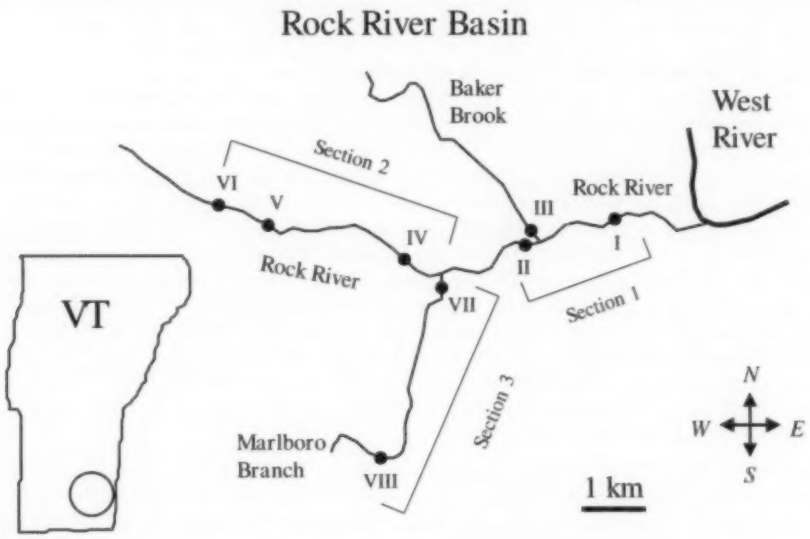
Fig. 1. Rock River Basin, Vermont (inset), and location of eight sites where snorkeling surveys were completed to quantify habitat availability and winter habitat selection of YOY and PYOY Atlantic salmon parr. The circle in the inset indicates the area of enlargement.



select different habitats in summer (Kennedy and Strange 1982) and during the fall–winter transition (Rimmer et al. 1984). It is unknown whether YOY and PYOY parr select different habitats in winter and thus whether they may be similarly susceptible to habitat limitations or potentially compete for preferred areas. For PYOY parr, winter habitat selection may also be influenced by fall maturation, a ubiquitous life history feature in male Atlantic salmon. Mature parr may exhibit greater movements from fall through winter (Whalen et al. 1999), which could influence winter habitat selection.

We determined the relationship between habitat availability and winter habitat selection of YOY and PYOY Atlantic salmon parr in a large southern Vermont tributary of the Connecticut River, U.S.A., that is subject to low winter water temperatures (≤1°C), variable and extensive icing, and periodic winter freshets. We addressed the following questions. (*i*) How does habitat selection of nocturnal Atlantic salmon parr relate to habitat availability in winter? (*ii*) Do YOY and PYOY parr select similar habitats? (*iii*) How does water temperature and ice conditions affect parr activity patterns? Our answers to these questions provide information on habitats potentially important to winter survival of Atlantic salmon parr as well as insight into the prospects for intraspecific competition for winter habitats.

Materials and methods

Study area

Habitat availability and habitat selection of Atlantic salmon parr were studied in the Rock River, a fourth-order tributary of the West River located in the Connecticut River Basin, U.S.A. (42°56′ N, 72°39′ W; Fig. 1). The Rock River has a basin area of about 155 km^2 and an average gradient of 1.7 m/km. Winter discharge is variable but estimated to range between 0.5 and 2.5 m^3/s. Atlantic salmon parr originate from unfed fry stocked annually in May at densities approximating 50/100 m^2, which typically results in age-1 parr densities of 3–10/100 m^2 (McMenemy 1995). The majority of smolts produced in the Rock River are age 2 (Whalen 1998).

We established eight 100-m sites distributed in upper and lower reaches of three sections of the Rock River Basin (Fig. 1). Sites ranged between 8 and 22 m in mean width and consisted primarily of riffle habitat dominated by cobble and rubble substrates (Crouse et al. 1981).

Parr habitat use

Parr habitat use was determined between November and March in the years 1995–1996 and 1996–1997 by night snorkeling. We divided the November–March winter period as follows: (*i*) November 21 to December 12, early winter; (*ii*) December 13 to February 15, midwinter; (*iii*) February 16 to March 28, late winter. We attempted to equally distribute our sampling effort among winter periods, but ice conditions in midwinter and turbid flows in late winter limited our sampling (Table 1).

Snorkeling procedures were described previously by Whalen et al. (1999). A diver entered the lower end of each site and moved upstream, searching both midchannel and stream margin habitats. Surveys were typically 1.5 h in length and were completed between 18:00 and 01:00. The position of undisturbed Atlantic salmon parr was marked with a numbered float. Parr <95 mm total length were designated as YOY and parr >95 mm total length were designated as PYOY. Previous sampling showed that few YOY parr are >95 mm total length and few PYOY parr are <100 mm total length by winter (K.G. Whalen and D.L. Parrish, unpublished data). Fish length was estimated using the method described by Baltz et al. (1991).

Behavior of parr upon encounter was recorded as resting, swimming, or feeding. We recorded the substrate type that parr were physically resting upon when encountered as silt–sand, gravel, cobble–rubble, boulder, or bedrock (Crouse et al. 1981). Anchor ice was also included as a substrate category. Additionally, we categorized the general habitat character of the position of each parr relative to cover that was defined as features of the physical habitat within about 0.5 m of parr providing protection from the stream

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Table 1. Overview of the study design used to evaluate habitat availability versus selection of YOY and PYOY Atlantic salmon parr.

		Winter period			
Section	Site	Early	Middle	Late	*N*
1	I	1	2	1	4
1	II	1	0	0	1
1	III	0	1	0	1
2	IV	1	1	1	3
2	V	1	0	0	1
2	VI	1	1	1	3
3	VII	2	4	1	7
3	VIII	1	0	1	2
	N	8	9	5	22

Note: The cell value is number of snorkeling surveys completed at each site within each winter period. The value in parentheses indicates the number of surveys completed during the day. *N* = number of surveys per site or per winter period. The locations of river sections and sites sre given in Fig. 1.

current. We established three "cover" habitat type categories defined as (*i*) dead zone: parr occupying quiescent habitats formed by the stream edge or large in-stream structure, e.g., boulders, woody debris, (*ii*) depositional: parr occupying the immediate downstream side of substrate, typically laterally compressed against the substrate, and (*iii*) surface ice: parr found beneath surface ice. We also established one "noncover" or "exposed" habitat type category defined as parr exposed to the current with no recognizable physical feature providing cover.

Upon completion of snorkeling, water depth (centimetres) and velocity (centimetres per second at 0.6 × depth) were recorded at the position of each parr as marked by the numbered float. To assess lateral distribution, we recorded distance to the nearest streambank and total stream width at each parr location. Before each snorkeling survey, water temperature was recorded with a hand-held thermometer and the percentage of the total surface area of the site covered by surface and anchor ice was visually estimated.

Habitat availability

To quantify available habitat during the variable ice conditions in early and midwinter, water depth and velocity were recorded at up to 50 randomly selected points within the study site immediately after snorkeling surveys were completed. We termed these measurements random available measurements (RAM). The procedure for RAM was as follows. Beginning at either the upper or lower end of the site, we used a random numbers table to generate *x*,*y*-coordinate pairs to be translated into "paces" by the observer. The *x*-coordinate was paced at a right angle to the river flow and the *y*-coordinate was paced parallel to the river flow. Water depth and water velocity at 0.6 × depth were recorded at the point defined by the randomly selected coordinates. After each measurement, new random coordinates were selected and the procedure was repeated through the entire length of the site. The RAM procedure enabled us to thoroughly, yet efficiently, characterize available water depths and velocities under the constraints of working in high flows and ice-laden rivers at night.

Parr maturity

As part of studies to determine the effect of parr maturity on smolt recruitment (Whalen and Parrish 1999), mature and immature PYOY Atlantic salmon parr were collected by electrofishing in October–November 1995 and 1996 and marked with either an acrylic paint injection to the anal fin (Whalen et al. 1999) or alcian blue dye applied with a panjet (Cunjak and Randall 1993). Additionally, some parr also received a passive integrated transponder (PIT) tag. Marked parr were observed during routine winter diving surveys. The fin paint injections enabled parr maturity to be determined during snorkeling without capturing parr. Other parr, collected using a small dip net, were placed into a water-filled metered tube and inspected for marks (Whalen et al. 1999). The maturity of captured unmarked parr was determined by applying gentle pressure to the vent area and parr freely expressing milt were deemed mature. Some mature parr continued to freely express milt through the end of March, about 5 months after the initial October–November maturation assessment.

Data and statistical analysis

We completed an initial screening analysis to determine the effects of section and winter period on habitat use and availability using analysis of variance (ANOVA). Greater differences in habitat use and availability were found among sections than among winter periods, so we focused on section-specific comparisons of YOY and PYOY parr water depth and velocity selection versus habitat availability (RAM). Additionally, we completed a summary multivariate analysis of habitat selection versus availability including all data collected over the two winters. We plotted water velocity (dependent variable) versus water depth (independent variable) for YOY and PYOY parr habitat selection as well as for RAM for habitat availability and then calculated a bivariate equal-frequency ellipse encompassing $100(1 - \alpha)\%$ of the observations, where $\alpha = 0.25$ (Sokal and Rohlf 1995; SAS Institute 1996). We plotted the equal-frequency ellipses to illustrate the overlap in YOY and PYOY parr habitat selection in relation to habitat availability and the specificity of parr habitat selection for water depth versus water velocity.

We used χ^2 analysis to compare substrate and habitat type use among YOY and PYOY parr. For substrate use, insufficient data were available for bedrock and anchor ice, so these categories were not included in statistical comparisons of substrate use among YOY and PYOY parr. Also, because few parr were found under surface ice, this category was not included in statistical comparisons of habitat type use among YOY and PYOY parr. Regression was used to determine if the number of YOY and PYOY parr observed in the area of the site surveyed was related to date. ANOVA was also used to determine if significant differences existed in average water temperature and ice coverage among winter periods.

We applied a $\ln(x + 1)$ transformation to YOY and PYOY parr water velocity selection data to correct for positive skewness and improve normality; nontransformed values are reported in the graphs and text. The Tukey–Kramer studentized multiple range test was used when a significant difference ($\alpha = 0.05$) among means was identified by ANOVA. Statistical significance for all analyses was considered at $\alpha = 0.05$.

Results

All snorkeling surveys were completed at water temperatures between 0 and 3°C. Mean water temperature tended to be colder in midwinter (0.4 ± 0.2°C) than in early (1.4 ± 0.3°C) and late winter (1.2 ± 0.3°C), but differences were not significant (ANOVA, $p > 0.05$). Average percent ice coverage was five- to 10-fold greater in midwinter (36 ± 11%) than in early (3 ± 2%) and late winter (7 ± 6%) (ANOVA, $p < 0.05$).

Over the 2 years, we completed 22 night snorkeling surveys, eight in early winter, nine in midwinter, and five in late winter (Table 1). Observations of nocturnal habitat use for parr were roughly equally distributed between YOY (N = 128) and PYOY (N = 127).

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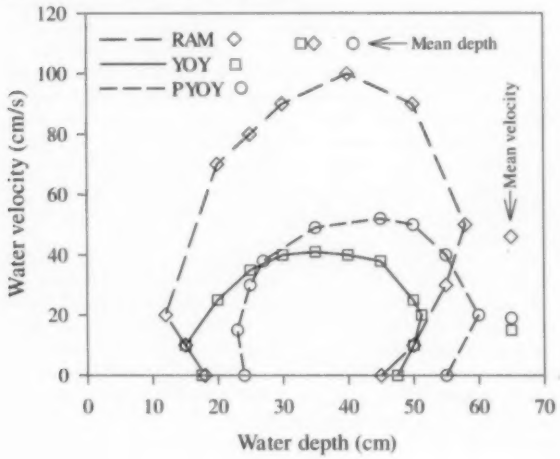
Table 2. Mean (±SE) water depth and water velocity selected by YOY and PYOY Atlantic salmon parr versus RAM for habitat availability by section (see Fig. 1).

	Water depth (cm)				Water velocity (cm/s)			
Section	YOY	PYOY	RAM	$p < 0.05$	YOY	PYOY	RAM	$p < 0.05$
1	34±2 (30) *a*	45±3 (23) *b*	40±1 (132) *ab*	***	12±3 (29) *a*	17±4 (21) *a*	57±3 (133) *b*	***
2	33±2 (31)	39±2 (33)	35±1 (224)	ns	12±3 (31) *a*	21±4 (33) *a*	44±2 (224) *b*	***
3	33±2 (41) *b*	41±1 (61) *a*	33±1 (254) *b*	***	19±3 (40) *a*	18±3 (61) *a*	44±2 (254) *b*	***

Note: Sample size is given in parentheses. The overall mean given in the last row is the weighted average. Results of within-site ANOVA: ***$p < 0.05$; ns, $p > 0.05$. Means not sharing a common letter are significantly different (Tukey–Kramer studentized range test).

Fig. 2. Bivariate plot of water depth versus water velocity for YOY (N = 99) and PYOY (N = 115) Atlantic salmon parr habitat selection and RAM (N = 610) recorded for habitat availability. Shown are representative points and lines defining the equal-frequency ellipses calculated to encompass 75% of the observations for each case. Overall univariate means for water depth and velocity are also plotted.



Habitat selection versus availability

Within each section, greater contrast between habitat selection of YOY and PYOY parr versus habitat availability existed for water velocity than for water depth (Table 2). PYOY parr tended to select deeper water than YOY parr as well as RAM, whereas both YOY and PYOY parr selected water velocities that were at least twofold lower than RAM (Table 2).

The bivariate summary of water depth versus water velocity illustrates the large degree of overlap in habitat selection of YOY and PYOY parr (Fig. 2). YOY and PYOY parr selected only a portion of available water velocities, whereas their water depth selection encompassed nearly the entire range of available water depths (Fig. 2). On the water depth axis, the PYOY parr ellipse extended beyond the RAM ellipse, indicating that some PYOY parr preferred water depths greater than those commonly available (Fig. 2).

Parr behavior and substrate and habitat type use

Behavior of YOY and PYOY parr upon encounter was very similar, as 97% of the 101 YOY and 96% of the 111 PYOY parr observed were resting in contact with the substrate. For both stages combined, only five parr were swimming upon encounter and only two were feeding. YOY and PYOY parr also used similar substrates (χ^2 = 5.0, $p >$ 0.05), with the majority found on silt–sand and gravel (Table 3). For both YOY and PYOY parr, the number of observations in each substrate category tended to increase with decreasing substrate size. YOY and PYOY parr were occasionally observed resting on anchor ice.

YOY and PYOY parr differed in habitat type use (χ^2 = 8.0, $p < 0.05$; Table 4). Most YOY parr were found in deadzone and depositional habitats, whereas observations of PYOY parr were nearly equally distributed among habitat types (Table 4). Generally, PYOY parr showed a greater tendency to be found in exposed habitats than YOY parr, a trend that had a temporal component. The proportion of observations of PYOY parr using exposed habitats decreased from 40% in early winter to 12% in late winter.

Parr were found close to the stream edge. The average stream width was 11 m at each YOY parr location and 12 m for PYOY parr. On average (±SE), distance from the stream edge was 2.2 ± 0.2 m for YOY parr and 2.6 ± 0.1 m for PYOY parr.

Seasonal activity patterns

We tested for the effect of year in a two-way ANOVA, including winter period for both the number of YOY and PYOY parr observed, to determine if pooling over years was possible. Year effects were not significant for PYOY parr (ANOVA, $p > 0.05$) but were significant for YOY parr (ANOVA, $p < 0.05$). Thus, to evaluate temporal trends in abundance for YOY parr, we used residuals of parr observations.

No relationship between residuals of the number of parr observed and date was found for YOY parr (regression, $p >$ 0.05). A significant relationship was found between number of PYOY parr observed and date (regression, $p < 0.05$, r^2 = 0.46; Fig. 3*a*). The number of PYOY parr observed was variable in all winter periods, decreasing from early winter to consistently low numbers through midwinter. The number of PYOY parr increased between midwinter and late winter but remained lower than numbers of parr observed in early winter. We plotted water temperature and percent ice coverage for each sample date to evaluate the influence of environmental factors on the number of PYOY parr observed, but no pattern between PYOY parr abundance and water temperature or percent ice coverage was evident (Fig. 3).

Parr maturity

Over the 2 years, we identified the state of maturity of 65 of the 127 PYOY parr observed: 29 mature and 36 immature. Mature and immature parr exhibited similarity in habi-

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Table 3. Number of YOY and PYOY Atlantic salmon parr using different substrates during night snorkeling surveys between November and March, 1995–1996 and 1996–1997.

	Silt–sand	Gravel	Cobble–rubble	Boulder	Bedrock	Anchor ice	Total
YOY	47 (46)	30 (29)	11 (11)	12 (12)	1 (1)	2 (2)	103
PYOY	38 (33)	46 (40)	16 (14)	10 (9)	2 (2)	2 (2)	114
Total	85 (39)	76 (35)	27 (12)	22 (10)	3 (1)	4 (2)	217

Note: Parenthetical values are percentages of the row total, except those in the bottom row, which are percentages of the column total.

Table 4. Number of YOY and PYOY Atlantic salmon parr using different habitat types during night snorkeling surveys between November and March, 1995–1996 and 1996–1997.

	Habitat type				
	Exposed	Dead zone	Depositional	Under surface ice	Total
YOY	18 (18)	36 (35)	47 (46)	1 (1)	102
PYOY	35 (32)	39 (36)	33 (31)	1 (1)	108
Total	53 (25)	75 (36)	80 (38)	2 (1)	210

Note: Parenthetical values are percentages of the row total, except those in the bottom row, which are percentages of the column total.

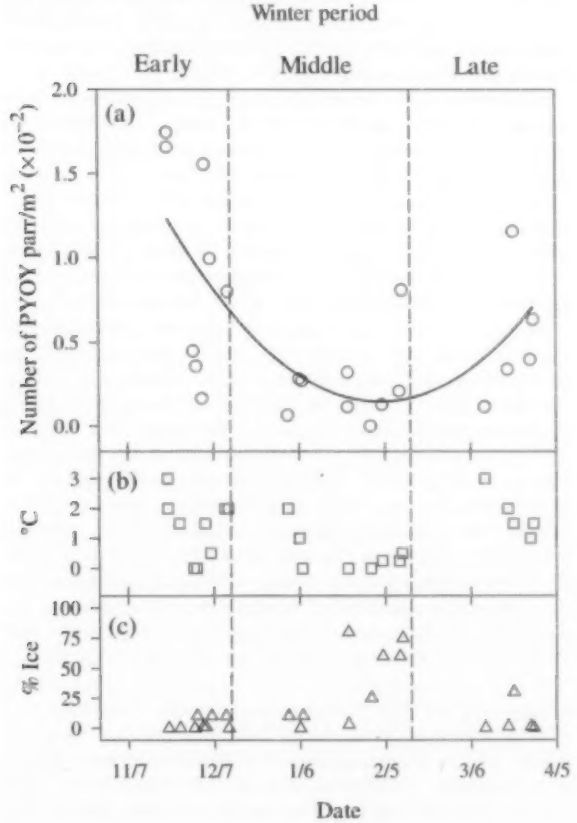

Fig. 3. Relationship of date to (*a*) number of PYOY Atlantic salmon parr observed, (*b*) water temperature, and (*c*) percent ice coverage recorded during 22 night snorkeling surveys. Broken vertical lines are the boundaries of the early-, mid-, and late-winter periods. The line in Fig. 3*a* is the fitted second-order polynomial ($p < 0.05$, $r^2 = 0.46$).

tat selection, as they selected similar water depths (ANOVA, $p > 0.05$) and velocities (ANOVA, $p > 0.05$). There was no evidence for differences among winter periods in the proportion of PYOY parr that were mature ($\chi^2 = 1.2$, $p > 0.05$), indicating no evidence for seasonal differences in activity pattern based on life history.

Discussion

Nocturnal YOY and PYOY Atlantic salmon parr exhibited specificity in habitat selection, with greater contrast between selection versus availability for water velocity than for water depth. The relationship between habitat selection and availability may provide clues to mechanisms by which changes in habitat could affect parr survival. The bivariate analysis of water depth versus water velocity showed that most YOY and PYOY parr exploited the full range, and beyond, of commonly available water depths, yet only a narrow range of available water velocities. The specificity that parr exhibit in selecting low water velocity habitats suggests that if these habitats are lacking or reduced during winter, parr survival may be reduced. The effect of changes in habitat availability on parr survival will ultimately depend on the amount of physical space parr require to feed and shelter. Measurements of how much habitat parr use are needed to determine how significant changes in habitat availability may be to winter survival when conditions become limiting.

PYOY parr occupied deeper waters and showed a tendency to use more exposed habitats than YOY parr, but generally, both PYOY and YOY parr exhibited similar preferences for low water velocity areas and most other elements of habitat selection. In differing sizes of nonanadromous brook (*Salvelinus fontinalis*) and brown trout (*Salmo trutta*), larger, older trout selected deeper water and at times higher water velocities than younger trout (Cunjak and Power 1986*a*, 1986*b*; Heggenes et al. 1993; Mäki-Petäys et al. 1997). As with nocturnal Atlantic salmon parr in winter, trout exhibited relatively stronger selection for water velocity than for water depth (Cunjak and Power 1986*a*; Mäki-Petäys et al. 1997). The similarity in YOY and

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PYOY parr habitat selection may be a response to a common environmental limiting factor such as low water temperature that may place limitations on activity independent of parr age (Graham et al. 1996).

The similarity in YOY and PYOY parr winter habitat selection has several consequences, e.g., (*i*) YOY and PYOY parr may be similarly susceptible to habitat limitations in winter and (*ii*) habitat limitations could result in competition for suitable areas. Several factors may ameliorate these effects, particularly the potential for interstage competition. Winter is a period of exceptional stochasticity in habitat conditions for juvenile Atlantic salmon (Cunjak and Randall 1993; Whalen et al. 1999). Continued disturbance of the physical habitat would limit the establishment of equilibrium conditions and the potential for competitive interactions (e.g., Hemphill and Cooper 1983). Typically, parr that we observed were distributed in patches, indicating the likelihood of competitive interactions was not strong. Trout that are territorial at other times of the year alter their behavior in winter and become tolerant of conspecifics, often aggregating in pools (Cunjak and Power 1986*b*). We observed no aggressive interactions among parr; rather, conspecifics were tolerant of others as close as 10–20 cm. A lack of aggressive interaction was also observed by Heggenes et al. (1993) and interpreted as an indication of a reduced focus on food acquisition in winter and, in turn, maintenance of territorial boundaries.

Our observations of parr habitat use are consistent with an overwinter strategy of minimization of net energy loss and basic maintenance rather than net energy gain and excessive activity (Heggenes et al. 1993). As reported for juvenile brown trout in the wild in winter (Heggenes et al. 1993) and for juvenile Atlantic salmon under winter-simulated conditions in the laboratory (Fraser et al. 1993), parr exhibited quiescent behavior, being often motionless upon encounter while resting in contact with the stream bottom. As observed for juvenile brown trout at night in winter (Heggenes et al. 1993), salmon parr were predominantly observed over fine-grained substrates, such as silt–sand and gravel, in dead-zone and depositional areas or habitats offering some recognizable cover from river flow. Where parr were found in higher flow areas, they often laterally compressed their body and fins against the downstream surface of the substrate. This behavioral regulation of position appeared to help minimize displacement by turbulent near-bottom flows. All features of Atlantic salmon parr winter habitat use that we identified, including behaviors presumably to resist displacement and minimize energetic demands, signify the importance of low water velocity refugia and thus the need to maintain large instream structure that provides cover from high flows and ice.

Parr were closely associated with the stream edge despite the presence of more ice in edge areas than in the midchannel. Flows under edge ice are minimal and when ice is not present, the stream edge provides refugia from high flows (Whalen et al. 1999). In contrast, the midchannel may be more often free of ice but subject to substrate scouring and higher flows (Erman et al. 1988; Whalen et al. 1999). We observed few salmon parr appearing to directly use surface ice as cover, which is in contrast with observations for trout where surface ice is a significant source of winter cover (Cunjak and Power 1986*b*). In our system, surface ice is labile relative to rocks and other forms of instream structure that provide more permanent flow refugia. The rarity of parr use of surface ice for cover may have been a consequence of the impermanence of ice rather than an indication of avoidance by parr.

The observation of fish sheltering in the substrate during the day in winter coupled with the observation of fish above the substrate at night has been interpreted as an adaptive response to capricious winter habitat conditions (Heggenes et al. 1993) as well as a predator avoidance mechanism (Valdimarsson and Metcalfe 1998). Atlantic salmon parr in the Rock River system are active at night at water temperatures ranging between 14 and 23°C in late summer (Gries et al. 1997). We have observed night-active salmon parr in all seasons and identified some similarities in habitat use and behavior at night in summer as observed here under winter conditions (K.G. Whalen and D.L. Parrish, unpublished data; see Metcalfe et al. 1997). Our findings are consistent with a laboratory analysis where there were differences in daytime sheltering of juvenile salmon but consistent nocturnal activity at water temperatures ranging from 2 to 18°C (Fraser et al. 1995). These results suggest that nocturnal activity in winter is a continuation of behavior exhibited at other times of the year rather than an adaptive response specifically for winter conditions. Because the adaptive or evolutionary significance of nocturnal activity will depend on the complete seasonal context within which this behavior is expressed, more research is needed to separate the effects of night versus day behavior from effects that may vary seasonally such as between summer and winter.

Maturity of PYOY parr had no significant effect on habitat use, which is consistent with the finding for mature and immature parr in West River tributaries to exhibit no significant difference in winter survival (Whalen 1998). In some cases, fall to spring survival of mature parr has been found to be substantially lower than that of immature parr (Myers 1984). Our results suggest that where such differences in winter survival of mature and immature parr occur, they may not be driven by differences in winter habitat selection.

The proportion of parr identified as mature (45%) at night was similar to that identified during daytime electrofishing surveys in the Rock River system (49%; Whalen and Parrish 1999). Mature and immature parr may differ energetically at the beginning of winter (Simpson 1992), and as a result of life history pathway, e.g., smolting versus nonsmolting, they may differ in behavior and exhibit dissimilar frequencies of nighttime emergence. A paradigm that has developed incorporating this theme is that upper modal fish (i.e., primarily immature parr expected to smolt the following spring) continue to feed and grow in winter, whereas lower modal fish (i.e., primarily mature parr expected not to smolt) enter a state of anorexia in winter and cease feeding (Metcalfe and Thorpe 1992). The similarity in percent mature that we observed during the day by electrofishing and at night by snorkeling suggests that mature and immature parr emerge at night at a frequency consistent with their relative frequency in the system. From our work, we can provide no evidence of a dichotomy in behavior or seasonal differences in activity for parr on smolting versus nonsmolting life history trajectories that have been observed under controlled condi-

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tions (Metcalfe and Thorpe 1992; Valdimarsson et al. 1997). Further analysis from different regions on winter activity patterns of mature and immature parr is warranted because of the significance of parr maturation to smolt recruitment (Myers 1984; Whalen and Parrish 1999).

The decline in the number of PYOY parr observed between early and midwinter could not be fully attributed to mortality because of the rebound in late winter to higher relative abundance. Heggenes et al. (1993) found a similar result for wild brown trout: late-winter (March) numbers of trout observed were higher than numbers observed during midwinter (January–February). Declines in relative densities were observed to accompany decreases in water temperature between fall and the onset of winter for juvenile rainbow trout (*Oncorhynchus mykiss*) (Riehle and Griffith 1993). Although the relative abundance pattern for PYOY parr was not related to water temperature or ice condition recorded on the sample date, average water temperatures in midwinter were significantly lower than those in early and late winter. In many instances, 7–10°C has been quoted as a threshold temperature that elicits behavioral responses, e.g., daytime sheltering, in juvenile salmon (Rimmer et al. 1983; Fraser et al. 1993). Small changes in water temperature that occur near the freezing point of freshwater, well below 7–10°C, could also cause behavioral changes and affect frequency of night emergence (Griffith and Smith 1993).

We hypothesize that differences in PYOY parr relative abundance over the winter may reflect behavioral changes related to the trade-off between the ability and need to meet a metabolic deficiency and the profitability and costs of activity. Unlike species of marine fish that have evolved to survive in chronically near-freezing and super-cooled conditions (DeVries 1988), Atlantic salmon have no known specialized mechanism for metabolically coping with near-freezing and super-cooled water. For subarctic brook trout, a compensatory physiological response occurs at 0°C where trout lower their metabolic demand, enabling them to survive for long periods without feeding (Cunjak and Power 1986*b*). Thus, less frequent activity may be necessitated at low water temperatures in midwinter because of lower metabolic demand, which is advantageous because low temperatures restrict swimming ability (Rimmer et al. 1985; Graham et al. 1996). Even though feeding may not be sufficient to offset the metabolic deficit occurring in winter (Cunjak and Power 1987), winter survival may be enhanced by acquiring some food rather than starving. We observed few instances of feeding by parr, but our sampling was not geared toward behavioral observations. Diet analysis on wild individuals has shown that Atlantic salmon parr continue to feed during winter, even in ice-covered streams at water temperatures near 0°C (Cunjak 1988). Although activity of salmon parr may be more infrequent in midwinter, the maintenance of foraging activity at water temperatures near 0°C may be an opportunistic behavior to offset an accumulated metabolic deficit.

In summary, Atlantic salmon parr exhibited specificity in nocturnal habitat selection. Differences in habitat quality among sites, specifically those habitats often selected by parr, could contribute to spatial variability in winter survival. YOY and PYOY parr were similar in most elements of behavior and habitat use, suggesting that both stages may be similarly susceptible to habitat limitations in winter. Generally, our observations of parr behavior and habitat use are consistent with a wintering strategy focusing on minimizing energetic loss rather than maximizing energetic gain. Habitat features such as large instream structure create low water velocity refugia that parr exploit to minimize exposure to high flows. Activity patterns of parr changed over the winter, and generally, fewer parr were observed in midwinter when water temperatures were at their minimum. The maintenance of activity at water temperatures near 0°C may reflect a trade-off between the need to feed to offset a metabolic deficit and the profitability and costs of activity. Further work to determine how the energetic condition of parr affects habitat use and survival would greatly contribute to the understanding of the effects of winter on recruitment success.

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References

Baltz, D.M., Vondracek, B., Brown, L.R., and Moyle, P.B. 1991. Seasonal changes in microhabitat selection by rainbow trout in a small stream. Trans. Am. Fish. Soc. **120**: 166–176.

Berg, N.H. 1994. Ice in stream pools in California's central Sierra Nevada: spatial and temporal variability and reduction in trout habitat availability. N. Am. J. Fish. Manage. **14**: 372–384.

Brown, R.S., and Mackay, W.C. 1995. Fall and winter movements of and habitat use by cutthroat trout in the Ram River, Alberta. Trans. Am. Fish. Soc. **124**: 873–885.

Crouse, M.R., Callahan, C.A., Malueg, K.W., and Dominguez, S.E. 1981. Effects of fine sediment on growth of juvenile coho salmon in laboratory streams. Trans. Am. Fish. Soc. **110**: 281–286.

Cunjak, R.A. 1988. Behavior and microhabitat of young Atlantic salmon (*Salmo salar*) during winter. Can. J. Fish. Aquat. Sci. **45**: 2156–2160.

Cunjak, R.A., and Power, G. 1986*a*. Seasonal changes in the physiology of brook trout, *Salvelinus fontinalis* (Mitchell), in a subarctic river system. J. Fish Biol. **29**: 279–288.

Cunjak, R.A., and Power, G. 1986*b*. Winter habitat utilization by stream resident brook trout (*Salvelinus fontinalis*) and brown trout (*Salmo trutta*). Can. J. Fish. Aquat. Sci. **43**: 1970–1981.

Cunjak, R.A., and Power, G. 1987. The feeding and energetics of stream-resident trout in winter. J. Fish Biol. **31**: 493–511.

Cunjak, R.A., and Randall, R.G. 1993. Instream movements of young Atlantic salmon (*Salmo salar*) during winter and early spring. Can. Spec. Publ. Fish. Aquat. Sci. No. 118. pp. 43–51.

DeVries, A.L. 1988. The role of antifreeze glycopeptides and peptides in the freezing avoidance of Antarctic fishes. Comp. Biochem. Physiol. B, Comp. Biochem. **90**: 611–621.

Erman, D.C., Andrews, E.D., and Yoder-Williams, M. 1988. Effects of winter floods on fishes in the Sierra Nevada. Can. J. Fish. Aquat. Sci. **45**: 2195–2200.

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Fraser, N.H.C., Metcalfe, N.B., and Thorpe, J.E. 1993. Temperature-dependent switch between diurnal and nocturnal foraging in salmon. Proc. R. Soc. Lond. Ser. B, Biol. Sci. **252**: 135–139.

Fraser, N.H.C., Heggenes, J., Metcalfe, N.B., and Thorpe, J.E. 1995. Low summer temperatures cause juvenile Atlantic salmon to become nocturnal. Can. J. Zool. **73**: 446–451.

Graham, W.D., Thorpe, J.E., and Metcalfe, N.B. 1996. Seasonal current holding performance of juvenile Atlantic salmon in relation to temperature and smolting. Can. J. Fish. Aquat. Sci. **53**: 80–86.

Gries, G., Whalen, K.G., Juanes, F., and Parrish, D.L. 1997. Nocturnal activity of juvenile Atlantic salmon (*Salmo salar*) at high water temperatures: evidence of diel activity partitioning. Can. J. Fish. Aquat. Sci. **54**: 1408–1413.

Griffith, J.S., and Smith, R.W. 1993. Use of winter concealment cover by juvenile cutthroat and brown trout in the South Fork of the Snake River, Idaho. N. Am. J. Fish. Manage. **13**: 823–830.

Heggenes, J., Krogg, O.M., Lindås, O.R., Dokk, J.G., and Bremnes, T. 1993. Homeostatic behavioral responses in a changing environment: brown trout (*Salmo trutta*) become nocturnal during winter. J. Anim. Ecol. **62**: 295–308.

Hemphill, N., and Cooper, S.D. 1983. The effect of physical disturbance on the relative abundances of two filter-feeding insects in a small stream. Oecologia, **58**: 378–382.

Kennedy, G.J.A., and Strange, C.D. 1982. The distribution of salmonids in upland streams in relation to depth and gradient. J. Fish Biol. **20**: 579–591.

Mäki-Petäys, A., Muotka, T., Huusko, A., Tikkanen, P., and Kreivi, P. 1997. Seasonal changes in habitat use and preferences by juvenile brown trout, *Salmo trutta*, in a northern boreal river. Can. J. Fish. Aquat. Sci. **54**: 520–530.

McMenemy, J.R. 1995. Survival of Atlantic salmon fry stocked at low density in the West River, Vermont. N. Am. J. Fish. Manage. **15**: 366–374.

Metcalfe, N.B., and Thorpe, J.E. 1992. Anorexia and defended energy levels in over-wintering juvenile salmon. J. Anim. Ecol. **61**: 175–181.

Metcalfe, N.B., Valdimarsson, S.K., and Fraser, N.H.C. 1997. Habitat profitability and choice in a sit-and-wait predator: juvenile salmon prefer slower currents on darker nights. J. Anim. Ecol. **66**: 866–875.

Myers, R.A. 1984. Demographic consequences of precocious maturation of Atlantic salmon (*Salmo salar*). Can. J. Fish. Aquat. Sci. **41**: 1349–1353.

Orciari, R.D., Leonard, G.H., Mysling, D.J., and Schluntz, E.C. 1994. Survival, growth, and smolt production of Atlantic salmon stocked as fry in a southern New England stream. N. Am. J. Fish. Manage. **14**: 588–606.

Power, G., Cunjak, R., Flannagan, J., and Kataopdis, C. 1993. Biological effects of river ice. *In* Environmental aspects of river ice. *Edited by* T.D. Prowse and N.C Gridley. National Hydrology Research Institute, Saskatoon, Sask. pp. 97–119.

Riehle, M.D., and Griffith, J.S. 1993. Changes in the habitat use and feeding chronology of juvenile rainbow trout (*Oncorhynchus mykiss*) in fall and the onset of winter in Silver Creek, Idaho. Can. J. Fish. Aquat. Sci. **50**: 2119–2128.

Rimmer, D.M., Paim, U., and Saunders, R.L. 1983. Autumnal habitat shift of juvenile Atlantic salmon (*Salmo salar*) in a small stream. Can. J. Fish. Aquat. Sci. **40**: 671–680.

Rimmer, D.M., Paim, U., and Saunders, R.L. 1984. Changes in the selection of microhabitat by juvenile Atlantic salmon (*Salmo salar*) at the summer–autumn transition in a small stream. Can. J. Fish. Aquat. Sci. **41**: 469–475.

Rimmer, D.M., Saunders, R.L., and Paim, U. 1985. Effects of temperature and season on the position holding performance of juvenile Atlantic salmon (*Salmo salar*). Can. J. Zool. **63**: 92–96.

SAS Institute. 1996. JMP® start statistics. *Edited by* J. Sall and A. Lehman. Duxbury Press, Pacific Grove, Calif.

Simpson, A.L. 1992. Differences in body size and lipid reserves between maturing and nonmaturing Atlantic salmon parr, *Salmo salar* L. Can. J. Zool. **70**: 1737–1742.

Sokal, R.R., and Rohlf, F.J. 1995. Biometry: the principles and practices of statistics in biological research. 3rd ed. W. H. Freeman and Company, New York.

Tschaplinski, P.J., and Hartman, G.F. 1983. Winter distribution of juvenile coho salmon (*Oncorhynchus kisutch*) before and after logging in Carnation Creek, British Columbia, and some implications of over-winter survival. Can. J. Fish. Aquat. Sci. **40**: 452–461.

Valdimarsson, S.K., and Metcalfe, N.B. 1998. Shelter selection in juvenile Atlantic salmon, or why do salmon seek shelter in winter? J. Fish Biol. **52**: 42–49.

Valdimarsson, S.K., Metcalfe, N.B., Thorpe, J.E., and Huntingford, F.A. 1997. Seasonal changes in sheltering: effect of light and temperature on diel activity in juvenile salmon. Anim. Behav. **54**: 1405–1412.

Whalen, K.G. 1998. Smolt production and overwinter mortality of Atlantic salmon (*Salmo salar*) stocked as fry. Ph.D. dissertation, University of Massachusetts, Amherst, Mass.

Whalen, K.G., and Parrish, D.L. 1999. Effect of maturity on parr growth and smolt recruitment of Atlantic salmon. Can. J. Fish. Aquat. Sci. **56**: 79–86.

Whalen, K.G., Parrish, D.L., and Mather, M.E. 1999. Effect of ice formation on selection of habitats and winter distribution of post-young-of-the-year Atlantic salmon parr. Can. J. Fish. Aquat. Sci. **56**: 87–96.

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Effect of velocity on the filter feeding of dreissenid mussels (*Dreissena polymorpha* and *Dreissena bugensis*): implications for trophic dynamics

Josef Daniel Ackerman

Abstract: Fluid dynamic forces were found to significantly affect the ability of freshwater dreissenid mussels (*Dreissena polymorpha* and *Dreissena bugensis*) to clear plankton. Tests conducted in a flow chamber at <1 cm·s^{-1} were consistent with published clearance rates from standard tests involving unstirred containers (i.e., 60–70 mL·mussel^{-1}·h^{-1} for 11-mm-long mussels). Increasing ambient velocity up to ~10 cm·s^{-1} led to clearance rates at least twice those of standard testing methods. Higher velocities (~20 cm·s^{-1}) were inhibitory and resulted in reduced clearance rates. There were no detectable differences in the clearance rates of *D. polymorpha* and *D. bugensis* of equal size tested at ~10 cm·s^{-1}, but large mussels had greater clearance rates than small ones. These results were found to be consistent with observations from marine bivalves and indicate that fluid dynamic issues are of importance in freshwater ecosystems, especially those that are shallow and (or) flowing. The trophic dynamics of these ecosystems will be better understood when the effects of fluid dynamics on the organism's ability to filter feed and the local delivery of seston through turbulent mixing are considered.

Résumé : Les forces en cause dans la dynamique des fluides ont un effet important sur la capacité des moules zébrées (*Dreissena polymorpha* et *Dreissena bugensis*) à retenir le plancton. Les résultats des tests menés dans une chambre d'écoulement à une vitesse inférieure à 1 cm·s^{-1} étaient conformes aux taux de rétention publiés à la suite d'épreuves standard effectuées à l'aide de contenants sans agitation (c.-à-d. 60–70 mL·moule^{-1}·h^{-1} pour des moules mesurant 11 mm de longueur). L'augmentation de la vitesse ambiante jusqu'à environ 10 cm·s^{-1} a permis d'obtenir des taux de rétention au moins deux fois plus élevés que les taux calculés par des méthodes d'essai ordinaires. Des vitesses plus élevées (environ 20 cm·s^{-1}) avaient un effet inhibiteur et abaissaient le taux de rétention du plancton. Les taux de rétention chez *D. polymorpha* et *D. bugensis* de même taille et à une vitesse d'environ 10 cm·s^{-1} ne présentaient aucune différence décelable, mais chez les moules de grande taille, le taux de rétention était plus élevé que chez les petites. Ces résultats étaient conformes aux observations faites chez des bivalves marins et montrent que les problèmes de dynamique des fluides ont une importance dans les écosystèmes d'eau douce, en particulier ceux qui sont peu profonds et/ou d'eau vive. On comprendra mieux la dynamique de la chaîne trophique de ces écosystèmes lorsque l'on prendra en compte les effets de la dynamique des fluides sur la capacité des organismes de filtrer la nourriture et sur le transport local du seston par mélange turbulent.

[Traduit par la Rédaction]

Introduction

In marine systems the importance of fluid dynamics in the transfer of energy from the water column to organisms on the seafloor has been recognized for some time (e.g., Mann and Lazier 1996). Recently, studies have revealed that tidal currents and turbulence affect the coupling of benthic and pelagic communities (Fréchette et al. 1989; Koseff et al. 1993; Butman et al. 1994; O'Riordan et al. 1995; Dame 1996; Wildish and Kristmanson 1997). Conversely, there has been less emphasis on fluid dynamic issues related to benthic–pelagic coupling in freshwater systems, which, with the exception of flowing systems, do not experience currents at regular intervals (Nixon 1988). These issues are, however, relevant to freshwater benthic suspension-feeding organisms including introduced zebra mussels (*Dreissena polymorpha*) and quagga mussels (*Dreissena bugensis*). The high population densities of dreissenid mussels (e.g., 10^5 mussels·m^{-2}) have led some researchers to conclude that the mussels are clarifying lakes and rivers in addition to their fouling of marine structures (MacIsaac et al. 1992; Nalepa and Schloesser 1993). While changes to phytoplankton species composition and water quality (e.g., phosphorus loading) have been reported in some shallow nearshore embayments (Holland 1993; Nicholls and Hopkins 1993; Fahnenstiel et al. 1995), little is known of the offshore and riverine environments

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Table 1. Response of bivalves to the experimental manipulation of water velocity.

Species	Habitat	Size (cm)	Testing device (dimensions, cm)	Flow rates ($cm \cdot s^{-1}$)
Argopecten irradians (Pectinidae)	Epifaunal nonsiphonate	3–4.5	Pipe (7.6 × 150)	0.17–12.8
		3–10	Flow chamber (30 × 11 × 200)	0–15
		0.8–1.4	Pipe (7.6 × 400)	0.7–14
Crassostrea gigas (Ostreidae)	Epifaunal nonsiphonate	4.80	Overflow chamber* (5.1 diameter)	0–0.16
Crassostrea virginica (Ostreidae)	Epifaunal nonsiphonate	5–8	Flow chamber (20 × 5 × 350)	0–4
Dreissena bugensis (Dreissenidae)	Epifaunal siphonate	1.1	Flow chamber (17 × 3.5 × 170)	0.4–19.4
Macoma spp. (Tellinidae)	Infaunal siphonate	3–7	Flow chamber (75 × 22 × 800)	0–40
Mercenaria mercenaria (Veneridae)	Infaunal siphonate	0.8	Overflow chamber* (5.1 diameter)	0–0.16
		4–5	Flow chamber (20 × 5 × 350)	0–4
Mya arenaria (Myidae)	Infaunal siphonate	3.40	Flow chamber (10 × 2.5 × 20)	<1–5.8
Mytilus edulis (Mytilidae)	Epifaunal nonsiphonate	3.00	Overflow chamber* (5.1 diameter)	0–0.16
		na	Overflow chamber	6×10^{-3} – 12 $mL \cdot s^{-1}$
		4.5–5	Flow chamber (15 × 19 × 80)	6–38
Ostrea edulis (Ostreidae)	Epifaunal nonsiphonate	4.40	Overflow chamber* (5.1 diameter)	0–0.16
Placopecten magellanicus (Pectinidae)	Epifaunal nonsiphonate	7.4–8	Flow chamber (20 × 25 × 500)	<1–90
		4–8	Flow chamber (20 × 25 × 500)	1.2–22.5
		7.3–8	Flow chamber (15 × 19 × 80)	6–44
Potamocorbula amurensis (Corbulidae)	Infaunal siphonate	0.5–2	Flow chamber (38 × 8 × 350)	9.5–25.3

Note: The Reynolds number (Re_{dh}) based on the hydraulic diameter provides an indication of the flow conditions in the testing device.
*Testing device dimensions (diameter × length or width × water depth × length) were approximated from figures in the original text.

(see Martel 1995). Potential impacts to these ecosystems have been extrapolated from standard laboratory results obtained in unmixed or aerated containers (e.g., Morton 1971; Sprung and Rose 1988; Horgan and Mills 1997) rather than from results obtained under mixed or dynamic flow conditions. The impacts of dreissenid mussels on aquatic ecosystems will require a characterization of their ability to filter seston (i.e., organic and inorganic fractions) under natural flow conditions (Nowell and Jumars 1984; Wildish and Kristmanson 1997).

There has been confusion as to the effects of velocity on marine bivalve feeding including both positive (feeding $\propto$ velocity) and negative effects (feeding $\propto$ 1/velocity) (Table 1). The former appear to be associated with studies that have been conducted under slow-flow and laminar conditions, while the latter are associated with faster and more turbulent conditions (e.g., *Mytilus edulis*) (Table 1), based on the Reynolds number (Re_{dh}) calculated for the test chamber (i.e., the hydraulic diameter, dh). Studies that were conducted over a wide range of velocities revealed that velocity has both positive effects under laminar conditions and negative effects under turbulent conditions (e.g., *Argopecten irradians*) (Table 1). In appears that velocity has a positive effect on feeding at very slow velocities and a negative or inhibitory one at higher velocities. This pattern has been called a "unimodal ramp-like function" and, more recently, a "continuous unimodal function" (Kirby-Smith 1972; Wildish and Kristmanson 1997). While the positive effects at low velocity may be due to factors such as the local replenishment of resources (see Peterson and Black 1987), the mechanisms responsible for feeding inhibition at higher flows are poorly understood (see Wildish and Kristmanson 1997).

Dreissenid mussels provide an important opportunity to examine whether, and under what circumstances, the afore-

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Re_{db}	Flow conditions	Feeding parameter	Velocity effects on feeding/growth	Study
$1.3 \times 10^2 - 9.7 \times 10^3$	Laminar, turbulent	Growth increment	Ramp function (inhibitory >5 cm·s^{-1})	Kirby-Smith 1972
$0 - 3.8 \times 10^4$	Laminar, turbulent	Growth rate	Ramp function (optimum ∝ [algae])	Calahan et al. 1989
$5 \times 10^2 - 1.1 \times 10^4$	Laminar, turbulent	Growth rate	Growth rate ∝ 1/velocity (inhibitory >9 cm·s^{-1})	Eckman et al. 1989
0–82	Laminar	Clearance rate	Clearance rate ∝ velocity	Walne 1972
$0 - 5.3 \times 10^3$	Laminar, turbulent	Growth rate	Ramp function (optimum ~1 cm·s^{-1})	Grizzle et al. 1992
$4.0 \times 10^2 - 1.9 \times 10^4$	Laminar, turbulent	Clearance rate	Ramp function (optimum <13 cm·s^{-1})	Present study
$0 - 2.2 \times 10^5$	Laminar, turbulent	Feeding radius	Feeding radius ∝ 1/velocity	Levinton 1991
0–82	Laminar	Clearance rate	Clearance rate ∝ velocity	Walne 1972
$0 - 5.3 \times 10^3$	Laminar, turbulent	Growth rate	Growth rate ∝ velocity	Grizzle et al. 1992
$67 - 3.9 \times 10^3$	Laminar, turbulent	Growth rate	Growth rate ∝ velocity	Emerson 1990
0–82	Laminar	Clearance rate	Clearance rate ∝ velocity	Walne 1972
na	Laminar	Pumping rate	Pumping rate ∝ velocity	Hildreth 1976
$1.3 \times 10^4 - 8.2 \times 10^4$	Turbulent	Clearance rate	Clearance rate ∝ velocity (inhibitory >25 cm·s^{-1})	Wildish and Miyares 1990
0–82	Laminar	Clearance rate	Clearance rate ∝ velocity	Walne 1972
$>0 - 2.6 \times 10^5$	Laminar, turbulent	Growth rate	Ramp function (inhibitory >20 cm·s^{-1})	Wildish et al. 1987
$3.4 \times 10^3 - 2.6 \times 10^5$	Turbulent	Growth rate	Growth ∝ 1/velocity	Wildish and Saulnier 1992
$1.3 \times 10^4 - 9.5 \times 10^4$	Turbulent	Clearance rate	Ramp function (optimum ∝ [algae])	Wildish et al. 1992
$2.1 \times 10^4 - 5.7 \times 10^4$	Turbulent	Clearance rate	Clearance rate ∝ 1/velocity (confounded by [algae])	Cole et al. 1992

mentioned velocity effects are relevant to benthic suspension feeders in freshwater ecosystems. The mussels are also interesting from the perspective of filter-feeding biology, as they possess both clam-like and mussel-like features. This is due to the fact that they are both epifaunal and siphonate (i.e., characteristics of mussels and clams, respectively), having originated from an infaunal, siphonate, clam-like ancestor (Morton 1993). Morton (1993) provided an excellent review of siphon morphology including (*i*) the more ventral and larger inhalant siphon with its numerous terminal tentacles and (*ii*) the more posterodorsal and smaller exhalant siphon, which lacks tentacles. Given that epifaunal bivalves do not usually have true extendable siphons (i.e., they may create pseudosiphons), it would be reasonable to ask whether the possession of extendible siphons affords dreissenid mussels advantages in their ability to obtain resources (c.f., Grizzle et al. 1992). More importantly, an examination of the filter feeding of dreissenid mussels should provide a better understanding of dreissenid mussel biology as well as insights into the role of fluid dynamics in the transfer of energy in freshwater ecosystems. The purpose of this paper is therefore to examine the influence of ambient velocity on the ability of dreissenid mussels (*D. polymorpha* and *D. bugensis*) to filter algae within a controlled environment of a laboratory flow chamber.

Materials and methods

The ability of different size-classes and species of dreissenid mussels to remove seston under flowing conditions was examined in a recirculating flow chamber. The experiments involved an examination of two size-classes of each species of dreissenid mussels (*D. polymorpha* (ROMIZ M8682) and *D. bugensis* (ROMIZ M8683), acquisition numbers of the Department of Invertebrate

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Fig. 1. Scale drawing of the recirculating flow chamber used in the study.

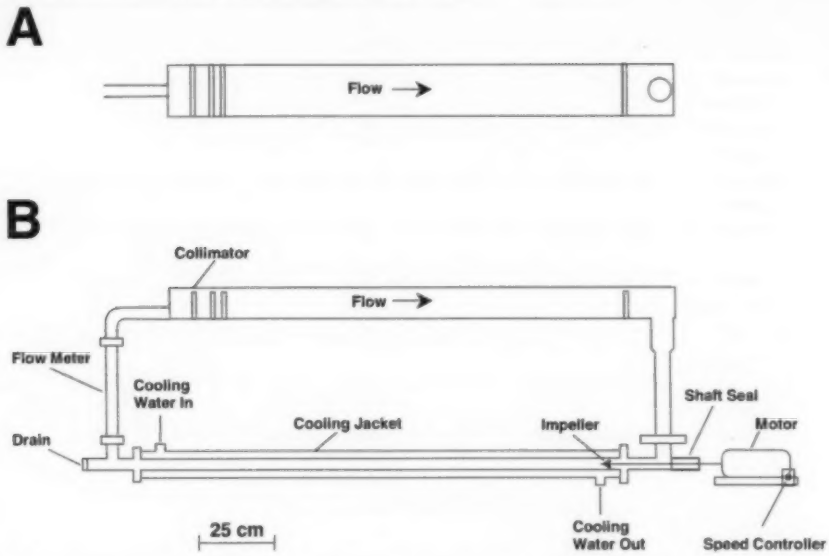

Table 2. Fluid dynamic conditions pertaining to the test conditions in the clearance rate experiments including the average velocity based on dye release studies (U), the chamber Reynolds number (Re_{dh} based on the hydraulic diameter) and flow conditions, the local Reynolds number (Re_x) at the downstream position of testing (i.e., x = 130 cm), and estimates of the friction velocity (u_*) based on laminar and turbulent flows (eqs. 7.25 and 7.44, respectively, in White 1994).

				Theoretical value of u_* (cm·s^{-1})	
U (cm·s^{-1})	Re_{dh}	Chamber flow conditions	Re_x	Laminar formulation	Turbulent formulation
0.40±0.01	4.0×10^2	Laminar	5.2×10^3	0.03	0.02
3.2±0.1	3.2×10^3	Turbulent	4.2×10^4	0.13	0.17
9.1±0.1	9.0×10^3	Turbulent	1.2×10^5	0.28	0.46
19.4±0.3	1.9×10^4	Turbulent	2.5×10^5	0.50	0.93

Zoology, Royal Ontario Museum, Toronto, Ont.; Rosenberg and Ludyanskiy 1994). Small *D. polymorpha* and *D. bugensis* (mean length ~11 mm for both species) were collected at Nanticoke, Ont., on Lake Erie, while large *D. polymorpha* and *D. bugensis* (mean length ~32 mm for both species) were collected at Cape Vincent, N.Y., near the St. Lawrence River. The mussels were transported to the laboratory and held under constant conditions in aquaria using filtered lake water maintained under constant filtration and aeration at 8–12°C and a 12 h light : 12 h dark cycle. Water changes were conducted regularly or as needed based on measured pH and oxygen levels. The mussels were provided with a mixture of live algae (*Chlorella* (UTCC90) and *Selenastrum* (UTCC37), University of Toronto Culture Collection, Department of Botany) on a regular basis.

The first set of experiments involved small *D. bugensis* with a mean (± SE) length of 11.4 ± 0.8 mm that had colonized concrete plates (8 × 15.5 × 0.25 cm) at a proportion of 96 ± 1% (the remaining 4% were *D. polymorpha*). There was an average density of 128 ± 13 mussels per plate (n = 3 plates), which is equivalent to ~10^4 mussels·m^{-2}. This is within the range of densities reported elsewhere but several orders of magnitude lower than the highest densities reported in Lake Erie (e.g., MacIsaac et al. 1992; Nalepa and Schloesser 1993). The remaining sets of experiments involved the examination of unattached mussels, which were tested separately according to species and size-class. Twenty individuals were examined in each trial involving small mussels (i.e., *D. polymorpha*), while five individuals were examined in each trial involving large mussels. The average lengths of the mussels examined were 11.0 ± 0.2 mm (small *D. polymorpha*), 32.5 ± 0.3 mm (large *D. polymorpha*), and 32.2 ± 0.5 mm (large *D. bugensis*).

Experiments were conducted in a rectangular flow chamber (17 cm wide × 10 cm deep × 170 cm long) with a 3.5-cm water depth (total chamber volume = 14.5 L) (Fig. 1). The water passed through three collimators (polyfibre material with honeycomb-shaped openings that were 4.7 cm wide × 3.2 cm high), positioned at 1, 5, and 10 cm from the inlet, before entering the testing region located 130 cm downstream of the last honeycomb. The outlet in the floor of the chamber was located 25 cm downstream of the testing region. Polyvinyl chloride pipe (5.3 cm diameter) was used to connect the outlet and inlet below the rectangular portion of the flow chamber. The flow in the chamber was generated by an axial flow pump and operated at a velocity of ~0.4, ~3.2, ~9, and ~19 cm·s^{-1}, as determined by dye release studies at midchannel and middepth (1.75 cm) at the testing location. Videography of egg white particles was used to profile the flow within the chamber.

The fluid dynamic conditions in the flume are presented in Table 2. They included (*i*) the measured average flow chamber velocity (U), (*ii*) the Reynolds number of the flow chamber (Re_{dh} based on the hydraulic diameter), which is a nondimensional ratio that provides an indication of whether the water flow is laminar or turbulent (Vogel 1994), (*iii*) the Reynolds number (Re_x) at the testing region, and (*iv*) estimates of the friction velocity (u_*) based on laminar and turbulent flows (eqs. 7.25 and 7.44, respectively, in White 1994). Velocity profiles for two convenient average chamber

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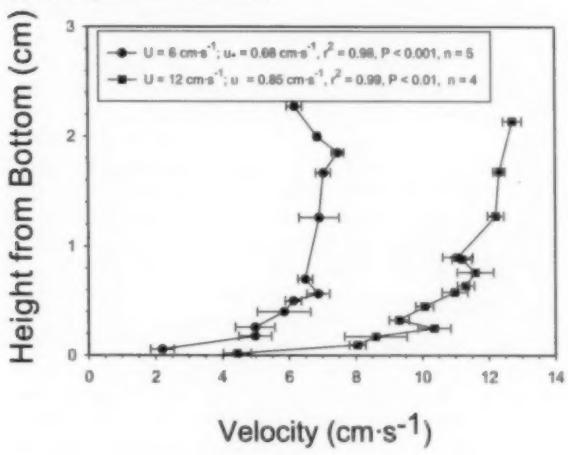
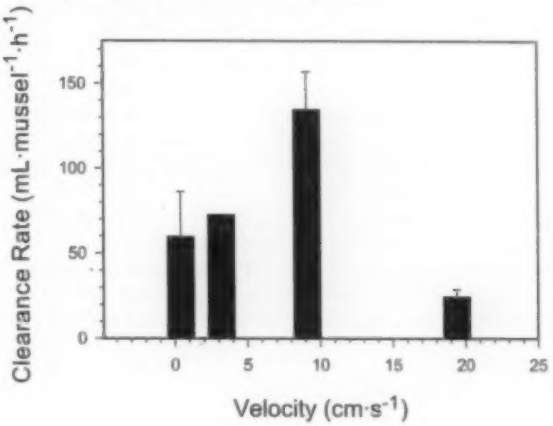
Fig. 2. Velocity profiles obtained from the recirculating flow chamber at the test position under 6 cm·s^{-1} (●) and 12 cm·s^{-1} (■) average chamber flow. The velocities (mean ± SE) were determined from the analysis of videotape recordings of boiled egg white particles in the flow.



velocities (6 and 12 cm·s^{-1}) were used to estimate u_*, which was based on von Karman's constant ($\kappa = 0.4$) times the slope of the regression of u on the natural logarithm of the height in the log layer (i.e., first five and four points in Fig. 2, respectively) (Nowell and Jumars 1984). The u_* provides an indication of the turbulent transfer and should be similar to u_* measured in the field to ensure the simulation of field conditions in the laboratory (Nowell and Jumars 1984; White 1994). The measured u_* values are much larger than the theoretical values (Table 2) determined from fluid dynamic theory for flow over a flat plate using the laminar approximation, yet somewhat similar using the turbulent approximation. The turbulent model is a reasonable one to use considering that the inlet flow immediately downstream of the collimators was turbulent, which would have allowed the velocity profiles to relax in the downstream direction to something approaching the 1/7 power law. This is in contrast with the conditions necessary for the flat plate approximation, which would include uniform inlet conditions where the boundary layers would develop in the downstream direction until they passed to the transition ($Re_x \sim 5 \times 10^5$) to turbulent flow (White 1994). Based on this arguments and Fig. 2, one could conclude that the experiments were conducted under fully developed boundary layer conditions, which would be found in the field where u_* values were measured to range between 0.25 and 2.1 cm·s^{-1} in Lake Erie (Bedford and Abdelrhman 1987).

The chamber was filled to a depth of 3.5 cm with water from Lake Erie filtered at 0.2 µm to remove natural food particles. Between 250 and 300 mL of a *Chlorella pyrenoidosa* (UTCC90) suspension of known concentration (300×10^3 to 2000×10^3 cells·L^{-1}) was added at the beginning of each experiment. Care was taken to ensure that the algae were well mixed in the chamber and that the chamber was operating at the average velocity, as defined above, before the introduction of mussels. Mussels (either on concrete plates or as individuals) were placed downstream (x = 130 cm) from the trailing edge of the honeycomb collimators (10 cm from the entrance). Unattached mussels were placed cross stream at x = 130 at a spacing of 0.5–1.0 cm with their inhalant siphons directed upstream. The starting time (time 0) of an experiment was defined when the valves opened and the siphons were extended, which was generally within ~5 min of the introduction of mussels. The water temperature varied by ≤1°C of the starting temperature (20.6 ± 0.3°C) over an experimental trial (i.e., 120 min).

Fig. 3. Effect of chamber velocity on the average clearance rate of *D. bugensis* (mean ± SE, n = 3, 1, 5, and 3).



Water samples (~5 mL) were taken at the entrance of the chamber and fixed immediately using two drops of Lugol's iodine acetic. The samples were stored at 4°C in the dark until being analyzed under a binocular microscope using a haemocytometer. Duplicate counts of cell numbers were made for each water sample, and the concentrations were provided as cells per millilitre. The change in the concentration through time was used to infer mussel clearance rates (i.e., volume of water filtered) using Coughlan's (1969) relationship:

$$(1) \qquad CR = \frac{V}{nt} \ln\left(\frac{C_0}{C_t}\right)$$

where CR is the clearance rate (millilitres per mussel per hour), V is the volume (millilitres), n is the number of mussels present, t is the period (hours), and C_0 and C_t are the algal concentrations (cells per millilitre) at the time 0 and time t, respectively. The observations reported below pertain to differences in algal concentration noted between t = 0 and 2 h, and so they should be interpreted as "average clearance rates" rather than instantaneous ones. Equation 1 assumes that there is no change in the algal concentration during an experiment, except for that removed by the suspension feeder, that the filtration rate of the bivalves is completely efficient, and that the rate of filtration is constant. The first assumption is a reasonable given the short duration (120 min) of the experiments, which likely precluded new algal production, and the smooth texture of the chamber surfaces, which minimized algal deposition. Subsequent experiments involving natural seawater from Bamfield Inlet (west coast of Vancouver Island) confirmed this by demonstrating that the loss of seston within the chamber in the absence of organisms was negligible (J.D. Ackerman, unpublished). The latter two assumptions are more difficult to resolve and will be addressed below.

Results

The average clearance rates of *D. bugensis* were strongly influenced by water velocity (Fig. 3) in a unimodal ramp-like manner or as a continuous unimodal function (Kirby-Smith 1972; Wildish and Kristmanson 1997). The mussels were able to filter feed at relatively high rates at velocities on the order of millimetres per second. Increasing velocity

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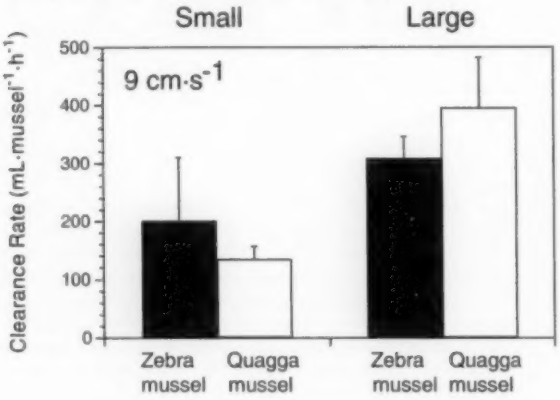
Table 3. *Dreissena bugensis* siphon extension/orientation related to ambient velocity.

	Test velocity ($cm \cdot s^{-1}$)				
Behavioral response	0.40	3.2	9.1	19.4	χ^2_3
Mussels with extended siphons	16	17	27	7	10.5*
Mussels gaped but siphons not extended	0	2	1	23	26.5*
Mussels with siphons redirected upstream	5	2	0	0	13.7*
Mussels observed on videotape recording	16	19	28	30	—

Note: The data were taken from videotape recordings made during the examination of mussel clearance rates.
*$P < 0.01$.

Fig. 4. Average clearance rates for small and large *D. polymorpha* (zebra) and *D. bugensis* (quagga) at a chamber velocity of ~9 $cm \cdot s^{-1}$ (mean ± SE, n = 3, 5, 3, and 3).



had a positive impact (i.e., more than double) on average clearance rate up to a velocity of ~9 $cm \cdot s^{-1}$. At some point beyond this, increasing velocity appeared to have an inhibitory effect leading to the lowest and most consistent (as indicated by the length of the standard error bars) average clearance rates at ~19 $cm \cdot s^{-1}$ (note that there was only a single observation at ~3.2 $cm \cdot s^{-1}$). An analysis of variance (ANOVA) revealed that there was a significant velocity effect on average clearance rate ($F_{3,8} = 4.68$, $P = 0.036$), and a priori pairwise comparisons (Fisher's least significant difference) revealed that there were significant pairwise differences between average clearance rates at ~0.4 and ~9 $cm \cdot s^{-1}$ and at ~9 and ~19 $cm \cdot s^{-1}$.

In addition to the effect of velocity on clearance rate, there were several behavioral observations pertaining to the orientation of inhalant and exhalant siphons, the position and angle of which were not fixed but could be modified by the mussels. While both inhalant and exhalant siphons are directed from the posterior of the mussels, the inhalant is located near the ventral margin, and the exhalant siphon is located at an angle toward the dorsal margin. The mussels were observed to modify the degree to which their siphons were extended and the direction in which they were directed relative to their orientation on the concrete plates. Unlike the orientation of siphons, the orientations of mussels were relatively fixed, since they were attached to the concrete plates. Videotape recordings of velocity experiments were used to determine (*i*) the number of mussels with extended siphons, (*ii*) the numbers of mussels that were gaped but did not have extended siphons, (*iii*) the numbers of mussels with siphons that were redirected upstream from a downstream-oriented mussel, and (*iv*) the numbers of mussels in which these data could be acquired unequivocally (Table 3) (only the middle one third of each concrete plate could be recorded). At ~0.4 $cm \cdot s^{-1}$, all of the mussels had extended siphons and 31% of these were redirected upstream. This situation was reversed at ~19 $cm \cdot s^{-1}$, where the majority of mussels (76%) gaped without extended siphons. Only 23% of these mussels had extended their siphons, albeit slightly, and none were redirected upstream. The situations at ~3.2 and ~9 $cm \cdot s^{-1}$ were similar to that at ~0.4 $cm \cdot s^{-1}$, although none of the mussels had redirected their siphons upstream at ~9 $cm \cdot s^{-1}$. The null hypothesis that the observed behaviors occurred in the same proportion at each velocity was rejected [$\chi^2 > \chi^2_{[3,0.01]} = 9.31$).

The remaining experiments, involving unattached mussels, were conducted at ~9 $cm \cdot s^{-1}$. It should be noted that the final position of individual mussels was scattered and several centimetres downstream of their initial and ordered placement at x = 130 cm. Mussel size appeared to have a strong influence on the rate at which mussels cleared *Chlorella* from the water (Fig. 4). The clearance rate for large mussels was ~1.5 to ~3 times the rate of small mussels. It should be noted, however, that the designation of "small" and "large" size categories pertains only to the mussels in this study, since the size range in nature is much greater (e.g., ~ 0.3 to ~50 mm; Ackerman et al. 1994). Statistically significant differences were not detected among the clearance rates of *D. polymorpha* and *D. bugensis* using ANOVA ($F_{1,12} = 0.074$, $P = 0.790$). Therefore, it was not possible to reject the null hypothesis that *D. polymorpha* and *D. bugensis* were similar in their ability to clear the water of algal cells. Significant differences were, however, detected between small and large mussels when species were pooled ($F_{1,12} = 9.91$, $P = 0.008$). Size differences were not detected between small and large *D. polymorpha* ($F_{1,4} = 0.873$, $P = 0.403$), due perhaps to the variation among individuals, but were found between small and large *D. bugensis* ($F_{1,6} = 13.96$, $P = 0.01$). One possible reason for this difference between species was the use of attached versus unattached mussels (the standard protocol used by most researchers), where lower values might be expected due to constant experimental conditions, crowding, and the inability to reorient and reposition in the former compared with the latter.

The relationship between average clearance rate and the algal concentration in the experiments reported here was weak or nonexistent (C_0 ranged from 15×10^3 to 340×10^3 $cells \cdot L^{-1}$). This can be seen in Fig. 5 where the average clearance rates for each experiment are plotted against the initial algal concentration for each species and size-class

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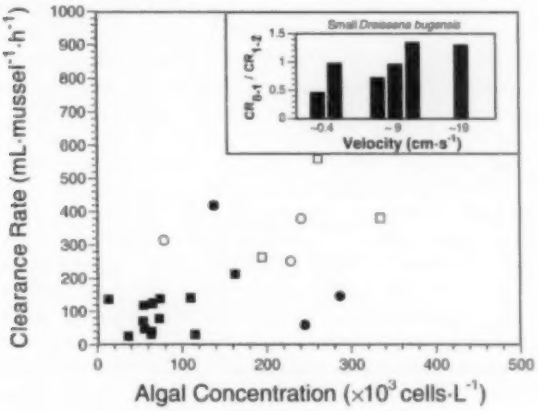
Fig. 5. Average clearance rates for small and large *D. polymorpha* (● and ○, respectively) and *D. bugensis* (■ and □, respectively) versus the initial algal concentration in the chamber. Open symbols represent large individuals of each species and solid symbols represent small individuals). Inset: quotient of the average clearance rate from 0 to 1 h (CR_{0-1}) and the average clearance rate from 1 to 2 h (CR_{1-2}) for six experiments involving small *D. bugensis* (first two bars, experiments conducted at 0.4 cm·s^{-1}; middle three bars, experiments conducted at 9.1 cm·s^{-1}; last bar, experiment conducted at 19 cm·s^{-1}).



separately. The situation is most evident where the data are plentiful as in the case of small *D. bugensis* (r = 0.41) (Fig. 5). The largest clearance rates are generally associated with the largest individuals of both species (Fig. 5) regardless of the initial algal concentration. The effect of declining seston concentrations during experimental trials (due to filter feeding) on mussel clearance rates is presented in the inset to Fig. 5. This inset presents the quotient of the average clearance rate from 0 to 1 h (CR_{0-1}) and the average clearance rate from 1 to 2 h (CR_{1-2}) for six experiments involving small *D. bugensis* in which clearance rates at 1 h were available (first two bars = 0.4 cm·s^{-1}, middle three bars = 9.1 cm·s^{-1}, last bar = 19 cm·s^{-1}). There does not appear to be a systematic difference between the first and the second hour of the experiment trials, with respect to the mussel's ability to clear seston from the chamber, nor does there appear to be a systematic bias in filtration rates among the treatments.

Discussion

Under very low velocities (i.e., ~0.4 cm·s^{-1}), the average clearance rates of small *D. bugensis* were generally in the range of 60–70 mL·mussel^{-1}·h^{-1}. These results are consistent with the range of values determined in previous studies of *D. polymorpha* and the one report pertaining to *D. bugensis* (see summary in Fig. 6). For example, Reeders et al. (1989) found that the clearance rate of *D. polymorpha* of different sizes, using various "food" (algae, clay particles) and experimental methods (laboratory, field), ranged from 2 to 287 mL·mussel^{-1}·h^{-1}. Kryger and Riisgård (1988) also reviewed these studies and characterized a size dependence on clearance rate, based on experiments involving *Chlorella* conducted in aerated containers (200 mL beakers). Their empirical relationship between clearance rate (CR) on algal cells and mussel size (shell length (SL) or tissue dry weight (DW)) is given by

$$(2) \qquad CR = 6.82(DW)^{0.88},$$

where $DW = 1.54 \times 10^{-5}\ (SL)^{2.42}$. Their model yielded a value of 68 mL·mussel^{-1}·h^{-1} for small mussels (i.e., 11.2 mm), which was consistent with the present results obtained at 0.4 cm·s^{-1}. The lower values in Fig. 6 were associated with the clearance of inert particles (e.g., graphite) or the movement of dye rather than algal cells.

The dependency of mussel size on clearance rate was consistent with other studies (e.g., Walz 1978; Kryger and Riisgård 1988; Wisniewski 1990; Horgan and Mills 1997), although the present results for large mussels were somewhat lower than Kryger and Riisgård's (1988) predicted rate of 658 mL·mussel·h^{-1} (32.5-mm mussel; see Fig. 6). However, it is possible that those results were overestimates because of experimental "wall effects." Wall effects occur when the siphonal currents of large mussels generate water currents within the confines of small test containers (200-mL beaker in the case of Kryger and Riisgård 1988), thus leading to high mixing rates that enhance clearance rates. The recognition of wall effects is supported by Wisniewski's (1990) rate of 260 mL·mussel^{-1}·h^{-1} for 31.1-mm-long mussels, which was obtained in large containers (2 and 5 L), where wall effects would be minimal. The present results showed a velocity effect when compared with Wisniewski's (1990) findings, although there may be separate ramp-like clearance rate velocity functions for different sizes of mussels.

There is considerable evidence that seston characteristics (e.g., type, algal species, concentration) affects mussel filter feeding (e.g., Ten Winkel and Davids 1982). Algal shape per se does not appear to affect clearance rates (Horgan and Mills 1997), although seston composition does. For example, inorganic and mineralogical particles generally lead to lower clearance rates (review in Kryger and Riisgård 1988), while some cyanophytes (blue-green algae) and colonial algae may be difficult to ingest and (or) sustain growth (Dorgelo 1993; but see Horgan and Mills 1997). In addition, seston concentration has an inverse relationship with clearance rate and a positive one with pseudofeces production (Walz 1978; Sprung and Rose 1988; Reeders and bij de Vaate 1992; Noordhuis et al. 1993). In this study, the limited range in initial algal concentrations precluded a "seston concentration effect" on mussel clearance rates (Fig. 5).

Velocity effects

Increased velocity in the chamber had a positive affect on clearance rate up to ~9 cm·s^{-1}, due likely to factors such as the replenishment of locally depleted resources (see review in Peterson and Black 1987). These latter results were among the highest clearance rate values determined for dreissenid mussels in the laboratory and values from the field (Noordhuis et al. 1993; Fig. 6). Further increases in velocity, however, led to declining clearance rates and would ultimately lead to the detachment of mussels through the breakage of byssal attachments at velocities of several metres per second (Ackerman et al. 1995).

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Fig. 6. Comparisons of clearance rates determined for small and large *D. polymorpha* and *D. bugensis* in this study and in the literature. Only those studies that examined mussels of 11 or 32 mm length, which is the size of mussels used in this study, were included in the comparison. The sources followed by asterisk indicate results pertaining to *D. bugensis*. Alimov (1969), Kondratév (1963), and Micheev (1966) are cited in Kryger and Riisgård (1988).

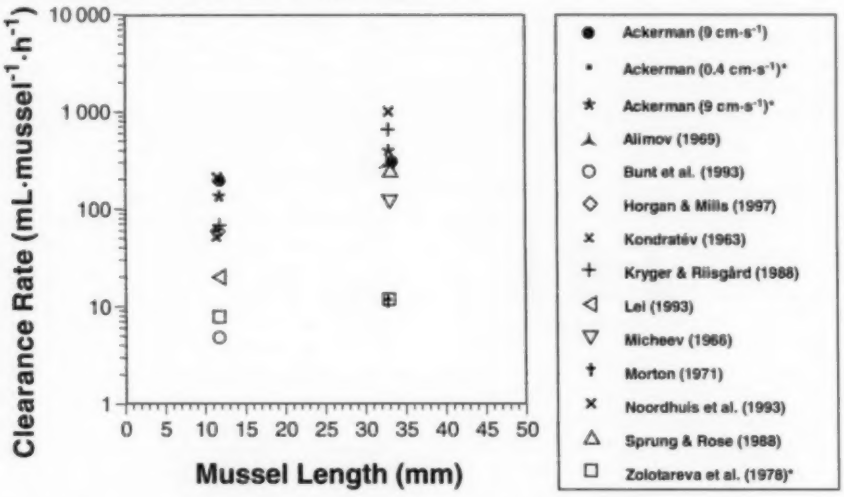

Table 4. Potential mechanisms responsible for reduced clearance rates at high ambient velocities.

Hypothesis	Mechanism	Behavioral/physiological response
Filtration instability	Positive pressure ($p_{inhalant} > p_{exhalant}$)	Change in siphon orientation, retraction, and closure/valve closure
	Negative pressure ($p_{inhalant} < p_{exhalant}$)	Disruption of bivalve pump and cessation of filter feeding
Behavioral instability	Lift/drag on mussel	Retract byssus/valve closure
	Lift/drag on siphons	Retract siphon/valve closure
Optimal foraging	Physiological control	Excess water shunted through gills; excess filtrate into pseudofeces

Note: In each case, a physical cue (i.e., p = pressure, lift, or drag) leads to a behavioral and (or) physiological response that reduces the clearance rate of the mussels.

There can be little support for the assertion that the observed velocity effects were due to the increased rate of recirculation, and hence the opportunity for mussels to filter feed on the seston in the chamber. If this were the case, one would predict that the highest clearance rates would be associated with the highest velocities in the chamber. This is opposite to what was observed in this study, where the lowest clearance rates were observed at the highest velocities in the chamber (Fig. 3). This would lead one to conclude that the effects are real, and it should be noted that the relationship of velocity with clearance rate is not observed and cannot be predicted from experiments using standard unmixed conditions in the laboratory.

The influence of velocity on filtration by marine bivalves has been recognized historically in anecdotal reports and experimentally since the 1970s (see Table 1). Bivalves can generate relatively large filtering currents as a result of the action of their gills (review in Jørgensen 1990), although without sufficient mixing, resources may become depleted and thus limit clearance rates (Peterson and Black 1987; Fréchette et al. 1989; Ackerman 1993). In other words, an increase in velocity and turbulent mixing has a positive effect on clearance rate of bivalves because of the replenishment of resources, although there may also be other factors involved (Wildish and Kristmanson 1997). This relationship peaks at some maxima for marine bivalves (Table 1), beyond which increased velocity is inhibitory to clearance rates (see below). The general velocity response (Fig. 3), with its ramp-like (Kirby-Smith 1972) or unimodal distribution (Wildish and Kristmanson 1997), has been observed in scallops, blue mussels, oysters, various clams, and now in freshwater dreissenid mussels (Table 1).

The mechanisms responsible for the inhibitory portion of the velocity response have yet to be delineated conclusively, although recent analyses by Wildish and Saulnier (1993) suggest three possibilities (Table 4): (*i*) filtration instability, in which velocity-induced dynamic pressure differentials (as small as 1–2 mm of water) under- or over-pressurize the inhalant and exhalant siphons (Wildish et al. 1987; Jørgensen 1990), (*ii*) behavioral instability, in which lift and drag forces acting at the scale of either a mussel or a siphon lead to shell closure and siphon retraction, respectively (e.g., Wildish and Kristmanson 1993; Levinton 1991), and (*iii*) optimal foraging, in which there is a physiologically optimal combination of seston concentrations and flow rates for efficient handling and processing of food (e.g., Calahan et al. 1989; Wildish et al. 1992). It was not possible to differentiate among the feeding-inhibition hypotheses for dreissenid mussels, nor is there reason to believe that they are mutually exclusive. The filtration instability hypothesis (positive pressure differentials) may be supported by the lack of extended siphons at high flow rates, but the calculated negative pres-

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sure differentials between inhalant and exhalant siphons were less than 1 mm H_2O (e.g., only 0.3 mm H_2O at ~19 cm·s^{-1} using the Bernoulli equation; White 1994). Behavioral instability likely occurred at the scale of both mussels and siphons, especially in terms of lift at ~20 cm·s^{-1}, siphonal Re corresponding to the production of Von Karman vortex streets (Re > 80 on circular cylinders; Vogel 1994), the lack of extended siphons at the highest flow (see Table 3), and the observed fluttering of filamentous algae and old byssal threads. The data were largely equivocal in the case of the optimal foraging hypothesis, but it should be noted that experiments were conducted at seston concentrations below the incipient threshold seston concentration for pseudofeces production in dreissenid mussels (Walz 1978; Sprung and Rose 1988; nominally 6.56×10^5 cells·L^{-1} for this study).

Unlike the marine bivalve systems that have been examined (Table 1), dreissenid mussels are simultaneously epibenthic and siphonate. This characteristic affords them a high degree of behavioral plasticity in terms of the orientation and extension of their siphons in filter feeding (Table 3). They appear to be capable of feeding under various combinations of siphon extensions and orientations. Under low flows, they can redirect their siphons into the flow and, through changes in the orientation and extension of their siphons, maintain relatively high clearance rates under relatively fast flows. Nonetheless, like other bivalve systems (Table 1), their ability to filter feed is reduced at higher flows.

Implications for trophic transfer in freshwater ecosystems

Fluid dynamic factors appear relevant to the ecology of freshwater bivalves in much the same manner as in marine bivalve systems. In the field, dreissenid mussels may be limited by the depletion of seston in the surrounding water due to their filter-feeding activity. This assertion is supported by flow visualizations that indicated that dreissenid mussels filter water within a small zone near the bottom (4–5 cm under a no-flow condition; Ackerman 1993). It is reasonable to conclude that without turbulent or wind-generated mixing delivering fresh seston, mussels would refilter the same volume of water. Increased velocity would thus replenish the depleted resources with fresh seston as a function of the ambient flow and mixing within the water column. One might expect this to occur in a manner similar to the positive relationship between velocity and clearance rate described above. Field studies indicate that marine mussels feed within a zone on the order of 3.5 cm, which can be related to the vertical and horizontal influences of siphonal currents (Muschenheim and Newell 1992). Physical modeling of siphonal flow in laboratory flumes is consistent with this and suggests that there may also be significant refiltration in bivalve beds (Monismith et al. 1990; O'Riordan et al. 1995). There are important implications for freshwater ecosystems, especially shallow waterbodies where wind-generated mixing would be enhanced and lotic or flowing habitats where enhanced flow would lead to greater impacts on the plankton.

Simple volumetric models of the impact of dreissenid mussels on the plankton, which use the quotient of the lake volume and the per unit area population clearance rate (i.e., population size distribution × size-corrected clearance rate per mussel), are of limited value except in shallow well-mixed conditions. This conclusion is supported by the fact that the physics of water flow and the effect of flow on mussel clearance rates are ignored. Models of the ecological impacts of dreissenid mussels and other benthic suspension feeders should incorporate these fluid dynamic effects on filter feeding in a benthic boundary layer model (e.g., Fréchette et al. 1989; Koseff et al. 1993; Butman et al. 1994; Wildish and Kristmanson 1997). While the results of this study provide important data to address this question using laboratory studies (i.e., flow chamber), more basic information is required. For example, the local flow (e.g., lentic versus lotic habitats, unidirectional versus oscillatory flow), mixing parameters (e.g., water currents, wind-generated waves, eddy viscosity), limnological morphometry (e.g., depth, surface area, bottom topography), and response of dreissenid mussels in situ should be measured. An evaluation of these data in a benthic boundary layer model would provide more realistic estimates of the ecological impacts of dreissenid mussels on freshwater ecosystems.

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References

Ackerman, J.D. 1993. Preliminary observations on the hydrodynamics of filter feeding in zebra mussels. *In* Bivalve filter feeders in estuarine and coastal ecosystem processes. *Edited by* R.F. Dame. Springer-Verlag, Berlin. pp. 513–514.

Ackerman, J.D., Sim, B., Nichols, S.J., and Claudi, R. 1994. A review of the early life history of zebra mussels (*Dreissena polymorpha*): comparisons with marine bivalves. Can. J. Zool. **72**: 1169–1179.

Ackerman, J.D., Cottrell, C.M., Ethier, C.R., Allen, D.G., and Spelt, J.K. 1995. A wall jet to measure the attachment strength of zebra mussels. Can. J. Fish. Aquat. Sci. **52**: 126–135.

Bedford, K.W., and Abdelrhman, M. 1987. Analytical and experimental studies of the benthic boundary layer and their applicability to near-bottom transport in Lake Erie. J. Gt. Lakes Res. **13**: 628–648.

Bunt, C.M., MacIsaac, H.J., and Sprules, W.G. 1993. Pumping rates and projected filtering impacts of juvenile zebra mussels (*Dreissena polymorpha*) in western Lake Erie. Can. J. Fish. Aquat. Sci. **50**: 1017–1022.

Butman, C.A., Fréchette, M., Geyer, W.R., and Starczak, V.R. 1994. Flume experiments on food supply to the blue mussel *Mytilus*

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edulis as a function of boundary-layer flow. Limnol. Oceanogr. **39**: 1755–1768.

Calahan, J.A., Siddall, S.E., and Luckenback, M.W. 1989. Effects of flow velocity, food concentration and particle flux on growth rates of juvenile bay scallops *Argopecten irradians*. J. Exp. Mar. Biol. Ecol. **129**: 45–60.

Cole, B.E., Thompson, J.K., and Cloern, J.E. 1992. Measurement of filtration rates by infaunal bivalves in a recirculating flume. Mar. Biol. **113**: 219–225.

Coughlan, J. 1969. The estimation of filtering rate from the clearance of suspensions. Mar. Biol. **2**: 356–358.

Dame, R.F. 1996. Ecology of marine bivalves. CRC Press, Boca Raton, Fla.

Dorgelo, J. 1993. Growth and population structure of the zebra mussel (*Dreissena polymorpha*) in Dutch lakes differing in trophic state. *In* Zebra mussels: biology, impact, and control. *Edited by* T.F. Nalepa and D.W. Schloesser. Lewis Press, Boca Raton, Fla. pp. 79–94.

Eckman, J.E., Peterson, C.H., and Calahan, J.A. 1989. Effects of flow speed, turbulence, and orientation on growth of juvenile bay scallops *Argopecten irradians concentrus* (Say). J. Exp. Mar. Biol. Ecol. **132**: 123–140.

Emerson, C.W. 1990. Influence of sediment disturbance and water flow on the growth of the soft-shell clam, *Mya arenaria* L. Can. J. Fish. Aquat. Sci. **47**: 1655–1663.

Fahnenstiel, G.L., Lang, G.A., Nalepa, T.F., and Johengen, T.H. 1995. Effects of zebra mussel (*Dreissena polymorpha*) colonization on water quality parameters in Saginaw Bay, Lake Huron. J. Gt. Lakes Res. **21**: 435–448.

Fréchette, M., Butman, C.A., and Geyer, W.G. 1989. The importance of boundary-layer flows in supplying phytoplankton to the benthic suspension feeder, *Mytilus edulis* L. Limnol. Oceanogr. **34**: 19–36.

Grizzle, R.E., Langan, R., and Howell, W.H. 1992. Growth response of suspension-feeding bivalve molluscs to changes in water flow: differences between siphonate and nonsiphonate taxa. J. Exp. Mar. Biol. Ecol. **162**: 213–228.

Hildreth, D.I. 1976. The influence of water flow on pumping rate in *Mytilus edulis* using a refined direct measurement apparatus. J. Mar. Biol. Assoc. U.K. **56**: 311–319.

Holland, R.E. 1993. Changes in planktonic diatoms and water transparency in Hatchery Bay, Bass Island area, western Lake Erie since the establishment of the zebra mussel. J. Gt. Lakes Res. **19**: 617–624.

Horgan, M.J., and Mills, E.L. 1997. Clearance rates and filtering activity of zebra mussel (*Dreissena polymorpha*): implications for freshwater lakes. Can. J. Fish. Aquat. Sci. **54**: 249–255.

Jørgensen, C.B. 1990. Bivalve filter feeding. Olsen and Olsen, Fredensborg, Denmark.

Kirby-Smith, W.M. 1972. Growth of the bay scallop: the influence of experimental water currents. J. Exp. Mar. Biol. Ecol. **8**: 7–18.

Koseff, J.R., Holen, J.K., Monismith, S.G., and Cloern, J.E. 1993. Coupled effects of vertical mixing and benthic grazing on phytoplankton populations in shallow, turbid estuaries. J. Mar. Res. **51**: 843–868.

Kryger, R.J., and Riisgård, H.U. 1988. Filtration rate capacities in 6 species of European freshwater bivalves. Oecologia, **7**: 34–38.

Lei, J. 1993. Estimation of filtration rate of zebra mussels. *Dreissena polymorpha* Inf. Rev. **4**(5): 8–9.

Levinton, J.S. 1991. Variable feeding behavior in three species of *Macoma* (Bivalvia: Tellinacea) as a response to water flow and sediment transport. Mar. Biol. **110**: 375–383.

MacIsaac, H.J., Sprules, W.G., Johannsson, O.E., and Leach, J.H. 1992. Filtering impacts of larval and adult zebra mussels (*Dreissena polymorpha*) in western Lake Erie. Oecologia, **92**: 30–39.

Mann, K.H., and Lazier, J.R.N. 1996. Dynamics of marine ecosystems. 2nd ed. Blackwell, Boston, Mass.

Martel, A. 1995. Demography and growth of the exotic zebra mussel (*Dreissena polymorpha*) in the Rideau River (Ontario). Can. J. Zool. **73**: 2244–2250.

Monismith, S.G., Koseff, J.R., Thompson, J.K., O'Riordan, C.A., and Nepf, H.M. 1990. A study of model bivalve siphonal currents. Limnol. Oceanogr. **35**: 680–696.

Morton, B.S. 1971. Studies on the biology of *Dreissena polymorpha* Pall. V. Some aspects of filter-feeding and the effect of micro-organisms upon the rate of filtration. Proc. Malacol. Soc. Lond. **38**: 301–321.

Morton, B.S. 1993. The anatomy of *Dreissena polymorpha* and the evolution and success of the heteromyarian form in the Dreissenoidea. *In* Zebra mussels: biology, impact, and control. *Edited by* T.F. Nalepa and D.W. Schloesser. Lewis Press, Boca Raton, Fla. pp. 185–215.

Muschenheim, D.K., and Newell, C.R. 1992. Utilization of seston flux over a mussel bed. Mar. Ecol. Prog. Ser. **85**: 131–136.

Nalepa, T.F., and Schloesser, D.W. (*Editors*). 1993. Zebra mussels: biology, impact, and control. Lewis Press, Boca Raton, Fla.

Nicholls, K.H., and Hopkins, G.J. 1993. Recent changes in Lake Erie (north shore) phytoplankton: cumulative impacts of phosphorus loading reductions and the zebra mussel introduction. J. Gt. Lakes Res. **19**: 637–647.

Nixon, S.W. 1988. Physical energy inputs and the comparative ecology of lake and marine ecosystems. Limnol. Oceanogr. **33**: 1005–1025.

Noordhuis, R., Reeders, H., and bij de Vaate, A. 1993. Filtration rate and pseudofaeces production in zebra mussels and their application in water quality management. *In* The zebra mussel *Dreissena polymorpha*. *Edited by* D. Neumann and H.A. Jenner. Gustav Fisher Verlag, Stuttgart, Germany. pp. 102–114.

Nowell, A.R.M., and Jumars, P.A. 1984. Flow environment of aquatic benthos. Annu. Rev. Ecol. Syst. **15**: 303–328.

O'Riordan, C.A., Monismith, S.G., and Koseff, J.R. 1995. The effect of bivalve excurrent jet dynamics on mass transfer in a benthic boundary layer. Limnol. Oceanogr. **40**: 330–344.

Peterson, C.H., and Black, R. 1987. Resource depletion by active suspension feeders on tidal flats: influence of local density and tidal elevation. Limnol. Oceanogr. **32**: 143–166.

Reeders, H., and bij de Vaate, A. 1992. Bioprocessing of polluted suspended matter from the water column by the zebra mussel (*Dreissena polymorpha* Pallas). Hydrobiologia, **239**: 53–63.

Reeders, H.H., bij de Vaate, A., and Slim, F.J. 1989. The infiltration of *Dreissena polymorpha* (Bivalvia) in three Dutch lakes with reference to biological water quality management. Freshwater Biol. **22**: 133–141.

Rosenberg, G., and Ludyanskiy, M.L. 1994. A nomenclatural review of *Dreissena* (Bivalvia: Dreissenidae), with identification of the quagga mussel as *Dreissena bugensis*. Can. J. Fish. Aquat. Sci. **51**: 1474–1484.

Sprung, M., and Rose, U. 1988. Influence of food size and food quality on the feeding of the mussel *Dreissena polymorpha*. Oecologia, **77**: 526–532.

Ten Winkel, E.H., and Davids, C. 1982. Food selection by *Dreissena polymorpha* (Mollusca: Bivalvia). Freshwater Biol. **12**: 553–558.

Vogel, S. 1994. Life in moving fluids. 2nd ed. Princeton University Press, Princeton, N.J.

Walne, P.R. 1972. The influence of current speed, body size and water temperature on the filtration rate of five species of bivalves. J. Mar. Biol. Assoc. U.K. **52**: 345–374.

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Walz, N. 1978. The energy balance of the freshwater mussel *Dreissena polymorpha* Pallas in laboratory experiments and in Lake Constance. Arch. Hydrobiol. Suppl. **55**: 83–105.

White, F.M. 1994. Fluid mechanics. 3rd ed. McGraw-Hill, New York.

Wildish, D.J., and Kristmanson, D.D. 1993. Hydrodynamic control of bivalve filter feeders: a conceptual view. *In* Bivalve filter feeders in estuarine and coastal ecosystem processes. *Edited by* R.F. Dame. Springer-Verlag, Berlin. pp. 299–324.

Wildish, D.J., and Kristmanson, D.D. 1997. Benthic suspension feeders and flow. Cambridge University Press, Cambridge, U.K.

Wildish, D.J., and Miyares, M.P. 1990. Filtration rate of blue mussels as a function of flow velocity: preliminary experiments. J. Exp. Mar. Biol. Ecol. **142**: 213–219.

Wildish, D.J., and Saulnier, A.M. 1992. The effect of velocity and flow direction on the growth of juvenile and adult giant scallops. J. Exp. Mar. Biol. Ecol. **155**: 133–143.

Wildish, D.J., and Saulnier, A.M. 1993. Hydrodynamic control of filtration in *Placopecten magellanicus*. J. Exp. Mar. Biol. Ecol. **174**: 65–82.

Wildish, D.J., Kristmanson, D.D, Hoar, R.L., DeCoste, A.M., McCormick, S.D., and White, A.W. 1987. Giant scallop feeding and growth responses to flow. J. Exp. Mar. Biol. Ecol. **113**: 207–220.

Wildish, D.J., Kristmanson, D.D, and Saulnier, A.M. 1992. Interactive effect of velocity and seston concentration on giant scallop feeding inhibition. J. Exp. Mar. Biol. Ecol. **155**: 161–168.

Wisniewski, R. 1990. Shoals of *Dreissena polymorpha* as bioprocessor of seston. Hydrobiologia, **200/201**: 451–458.

Zolotareva, V.I., Makhonina, A.V., and Dyga, A.K. 1978. The filtration rate of *Dreissena bugensis* Andrusov. Malacol. Rev. **11**: 96–97.

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Disentangling the effects of size-dependent encounter and susceptibility to predation with an individual-based model for fish larvae

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Abstract: We investigated the effects of size-dependent encounter and susceptibility, the role of variation in the size distribution of predators, and the timing of prey–predator interaction during the larval phase in shaping the length frequency distribution of surviving fish larvae. These analyses based on general empirical size-dependent relationships may have broad implications in understanding larval fish cohort dynamics. We demonstrated that the formulations of encounter and susceptibility to predation counteract each other, an increased range of predator sizes reduces only slightly the evidence for size-selective mortality, and synchronous spawning and hatching events have the potential to yield strong size-selective mortality of a cohort of fish larvae. The important factors in generating size-selective mortality are either the timing of encounters between fish larvae and their predators or high mortality rates. We demonstrated a direct relationship between the potential of size-selective mortality and the overall mortality rate of the cohort. We suggest that it may be difficult to detect the effect of size-dependent processes in the field. A better understanding of the factors influencing encounter represents a critical element in extrapolating laboratory studies of predation to the field.

Résumé : Nous avons évalué les effets de rencontre et susceptibilité (tous deux spécifiques aux tailles des proies et des prédateurs), le rôle de la variation de taille des prédateurs et l'effet du degré de synchronisme de l'intéraction proie–prédateur pendant le stage larvaire sur la distribution de tailles des larves de poissons. Ces analyses basées sur des relations empiriques générales pourraient avoir des conséquences globales pour comprendre les dynamiques des cohortes de larves de poisson. Nous avons démontré que les fonctions de rencontre et de susceptibilité à la prédation se neutralisent, qu'une augmentation de la gamme de tailles des prédateurs ne réduit que très peu la mortalité sélective des larves et qu'une synchronisation du frai et de l'éclosion a le potentiel de générer une importante mortalité sélective des tailles. Les facteurs importants pour générer une mortalité sélective sont une synchronisation des rencontres entre les larves de poissons et leurs prédateurs et un haut taux de mortalité. Nous démontrons une relation directe entre le potentiel sélectif de mortalité et la mortalité totale de la cohorte des poissons larvaires. Nous suggérons qu'il peut être difficile de détecter les effets de processus sélectifs sur le terrain. Une compréhension plus exhaustive des facteurs influençants les taux de rencontres est un élément clé pour l'extrapolation des études de prédation en laboratoires sur le terrain.

Introduction

Predation is a major source of mortality during the early life stages of fishes (Houde 1987; Bailey and Houde 1989). A prey–predator interaction is the sequence of three events: encounter between a prey and a predator, attack by the predator, and capture of the prey (Greene 1986). The sizes of both prey and predator are important factors determining the outcome of predation interactions (e.g., Werner and Gilliam 1984; Bailey and Houde 1989; Paradis et al. 1996). Analyses based on general patterns of encounter and susceptibility (where the latter is the combination of attack and capture) may reveal qualitative results with broad implications (Rice et al. 1997). In previous individual-based models, the relationships for either attack or capture were derived from single experimental studies or from species-specific observations (e.g., Rice et al. 1993*a*, 1993*b*, 1997; Cowan et al. 1996, 1997; Letcher et al. 1996). Paradis et al. (1996) compared several laboratory experiments on predation of fish larvae by different types of predators and showed that the

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Table 1. Characteristics of the size frequency distributions of crustacean, ctenophore, pelagic fish, and medusa predators calculated with the size ratio of Paradis et al. (1996) and drawn at random with a random number generator, and characteristics of the initial fish larvae length distribution and growth rate distribution.

	Mean	SD	Minimum	Maximum
Size frequency (mm)				
Crustaceans	10	5	1	20
Ctenophores	20	10	1	40
Pelagic fishes	185	80	15	355
Medusae	55	27	1	110
Fish larvae length (mm)	3	0.9	1	5
Fish larvae growth rate (mm·day^{-1})	0.5	0.08	0.2	0.8

laboratory conditions of these experiments (e.g., temperature, container volume, and duration of the experiment) could significantly influence the pattern of size-selective predation rates and the magnitude of predation mortality.

Previous individual-based models have contributed significantly to our understanding of the mechanisms by which predation mortality influences survival during the early life stages of fish. Rice et al. (1993*a*) found that faster growing cohorts of fish larvae had a higher survival rate than slower growing cohorts and that the size distribution of survivors was generally larger than expected from considerations of growth rates alone. Rice et al. (1993*b*) demonstrated that choosing a different size-class of predator can produce the opposite effect of size-selective predation on the size distribution of survivors. Cowan et al. (1996), by combining variations in the parameters of the growth rate distribution as well as predator size structure and foraging strategies, also found that faster growing cohorts of fish larvae had a higher survival rate than slower growing cohorts. Cowan et al. (1996) further demonstrated that size-selective mortality was apparent only after a significant number of fish larvae died (~70% of the original cohort).

Cowan et al.'s (1996) results are conditional on the size-class of predators (large versus small) that they examined. They modeled different size-classes of predators separately and compared foraging patterns of large and small predators. Rice et al. (1993*b*) and Letcher et al. (1996) have argued that it is the full range of predator sizes that is an important factor in shaping the size distribution of survivors. Rice et al. (1997) further developed their model by incorporating growth rate and size distributions for predators. Fish larvae and predators were growing at differential rates such that fish larvae could still outgrow the predator's preferred prey size. They found that small changes in these factors had strong effects on the size distribution of surviving fish larvae. Most important was the demonstration of how these effects change over time. Rice et al. (1997) argued that strong selection in one direction late in an interaction may completely erase evidence of strong selection in the opposite direction occurring at earlier stages, leaving the false impression that no size selection was occurring at all.

In this study, we extend previous models to investigate the role of variations in predator size and abundance in shaping the length frequency distribution of surviving fish larvae. We also investigate the influence of different timings of prey–predator interactions and the effects of encounter and susceptibility in shaping the length frequency distribution of surviving fish larvae. We compare these results for four different predator types: crustaceans, ctenophores, medusae, and pelagic fishes.

Materials and methods

Model description

The model was designed to simulate the growth and predation mortality of fish larvae for 30 days. The initial cohort of fish larvae was composed of 10 000 individuals, where each larva was characterized by a specific and discrete length and growth rate (Table 1) drawn at random from a normal distribution (Devroye 1986).

Fish larvae were subject to predation by four types of predators: crustaceans, ctenophores, medusae, and pelagic fishes. The size distribution of each predator was generated from a normal distribution (Devroye 1986). The minimum and maximum sizes of predators were restricted (Table 1) so that the relative size of fish larvae to predator remained within the range of previous laboratory studies (see review by Paradis et al. 1996). This was possible because predators were not allowed to grow during the simulation run. Predator size referred to the length of crustaceans and pelagic fishes and the diameter for ctenophores and medusae.

Each individual fish larva was confronted with predation by one of the four predator populations each day for 30 days. We evaluated whether the larva encountered a predator and whether it was captured by the predator. The predation model of this study was a combination of three processes: encounter between fish larvae and predator, susceptibility of fish larvae to the encountered predator, and growth of survivors. Encounter rates were calculated according to the Gerritsen and Strickler's (1977) equation modified to account for nonzero prey sizes (Bailey and Batty 1983). Susceptibility probabilities were based on the general laboratory-derived predation equations of Paradis et al. (1996). Growth of fish larvae was based on the constant-growth model of Rice et al. (1993*a*).

The individual-based model presented in this study is similar to Cowan et al.'s (1996) individual-based model. However, we explicitly incorporated the size of predator as an important factor determining the outcome of predation, as suggested by Rice et al. (1993*b*). The susceptibility probability was a function of both prey and predator sizes and was based on multispecific empirical relationships for the four predator types (Paradis et al. 1996) whereas the susceptibility probability of Cowan et al. (1996) was a function of prey size only and was based on laboratory measurements of realized capture probability (from Cowan and Houde 1992).

Encounter model

The encounter rates (E, encounters per day) between a fish larva and a predator were based on a modification of the Gerritsen and

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Table 2. Values of the parameters of the equations of encounter radius, swimming speed, and susceptibility (eqs. 2, 3, and 5, respectively).

			Predator			
Equation	Parameter	Fish larvae prey	Crustaceans	Ctenophores	Medusae	Pelagic fishes
Encounter radius	a	$2/\pi^2$ [v]	0.1[w]	0.5[x]	0.5[x]	0.8[x]
Swimming speed	b	0[y]	0[w]	0[z]	1.2[z]	0[y]
	c	1.0[y]	5.0[w]	1.16×10^{-6} [z]	0.4[z]	1.0[y]
	d	1[y]	1[w]	1.22[z]	1[z]	1[y]
Susceptibility	g	na	−5.6±0.4	−6.1±0.8	−7±4	−0.25±1
	h	na	−1.0±0.2	−2.1±0.4	−1.0±0.2	−1.9±0.6
	i	na	0	0.5±0.1	0	−0.4±0.1

[v]Bailey and Batty (1983).
[w]Lillelund and Lasker (1971), von Westernhagen et al. (1979), Greene (1986), and Bailey and Houde (1989).
[x]Cowan et al. (1996).
[y]Ware (1975).
[z]Cowan and Houde (1992).

Strickler (1977) model for randomly moving organisms in a three-dimensional space such that

$$(1)\quad E = \pi \times D \times C \times A_p \times R_{total}^2 \times \left(\frac{V_f^2 + 3 \times V_p^2}{3 \times V_p} \right) \text{ for } V_p \geq V_f$$

$$E = \pi \times D \times C \times A_p \times R_{total}^2 \times \left(\frac{V_p^2 + 3 \times V_f^2}{3 \times V_f} \right) \text{ for } V_p \leq V$$

where V_f and V_p were the swimming speeds (millimetres per second) of prey and predator, respectively), A_p was the abundance of predators (predators per cubic metre), D was the proportion of daylight hours in a day (0.54, based on 13 h·day^{-1}), and C was a conversion factor (8.64×10^4 s·day^{-1}). Originally, Gerritsen and Strickler (1977) defined an encounter radius independent of the sizes of organisms. The total encounter radius (R_{total}, millimetres) was the sum of the encounter radii of fish larvae and predator such that the encounter radius of prey or predator was defined as

$$(2)\quad R = a \times L$$

where L was either the length of fish larvae (L_f, millimetres) or the size of predator (L_p, millimetres) and a was a parameter (Table 2). Equation 2 allowed us to relax the original assumption that the animals are dimensionless points in space (Bailey and Batty 1983). The swimming speeds of fish larvae and predator (V) were also length dependent and were calculated as

$$(3)\quad V = b + c \times L^d$$

where b, c, and d were parameters (Table 2).

We allowed several encounters between a prey and a predator by calculating the probability of several encounters ($P(N)$) with a standard Poisson distribution as follows:

$$(4)\quad P(N) = \frac{(E \times t)^N \times e^{-E \times t}}{N!}$$

where N is the number of possible encounters and t is 1 day.

Each day and for each larva, we determined at random the numbers of encounters with a predator (N). In addition, we determined the size of the predator encountered (L_p) with another random number, based on the normal size frequency distribution of the predator population.

Susceptibility model

Susceptibilities (S) were derived from the laboratory-corrected predation equations of Paradis et al. (1996) for specific predator types such that

$$(5)\quad \ln(S) = g + h \times \ln \frac{L_f}{L_p} + i \times \left(\ln \frac{L_f}{L_p} \right)^2$$

where g, h, and i were parameters (Table 2). The parameter g included the laboratory corrections calculated by Paradis et al. (1996). Under average laboratory conditions, container volumes are small and duration of an experiment is short. Under these conditions, encounter rates between all four types of predators and fish larvae are greater than 1, if they are calculated with Gerritsen and Strickler's (1977) equation (see Appendix). This implies that the prey and predator under average laboratory conditions are contained within the same body of water. We assumed that on the larger spatial (thousands of cubic metres of water) and temporal (30 days) scales as in our simulation model, encounter rates are not equal to 1. Therefore, a predation rate calculated under laboratory conditions represented susceptibility under field conditions (see Appendix).

Each day and for each larva, we used a random number to determine which of N encounter(s) lead to a capture given that we had determined the length of predator encountered.

Vulnerability curves

In our model, we defined encounter and susceptibility as functions of both prey and predator sizes. The product of these equations is vulnerability. The model predicts that small fish larvae will be most vulnerable to all three invertebrate predators whereas larger fish larvae will be most vulnerable to predation by pelagic fishes of intermediate sizes (Fig. 1). Specifically, only the smallest fish larvae are most vulnerable to the largest crustaceans or medusae (Figs. 1*a* and 1*c*). Changes in vulnerability of larval fishes to predation by pelagic fishes are mostly governed by variations of predator sizes (Fig. 1*d*). The range of fish larvae most vulnerable to predation by ctenophores increases with ctenophore sizes (Fig. 1*b*). Furthermore, the peak vulnerability changes given different sizes of predators and occurs for similar length of fish larvae (Fig. 1). It is obvious that including predator sizes at all stages of a predation interaction (encounter and susceptibility) may lead to different conclusions about the effects of a predator population on the survival rates and the characteristics of surviving fish larvae.

Growth model

Each larva was assigned a growth rate (millimetres per day) drawn at random from a normal distribution (Table 1), and its assigned growth rate was maintained throughout the entire simulation. At the end of each day, the lengths of all surviving individuals were updated with the individual's growth rate.

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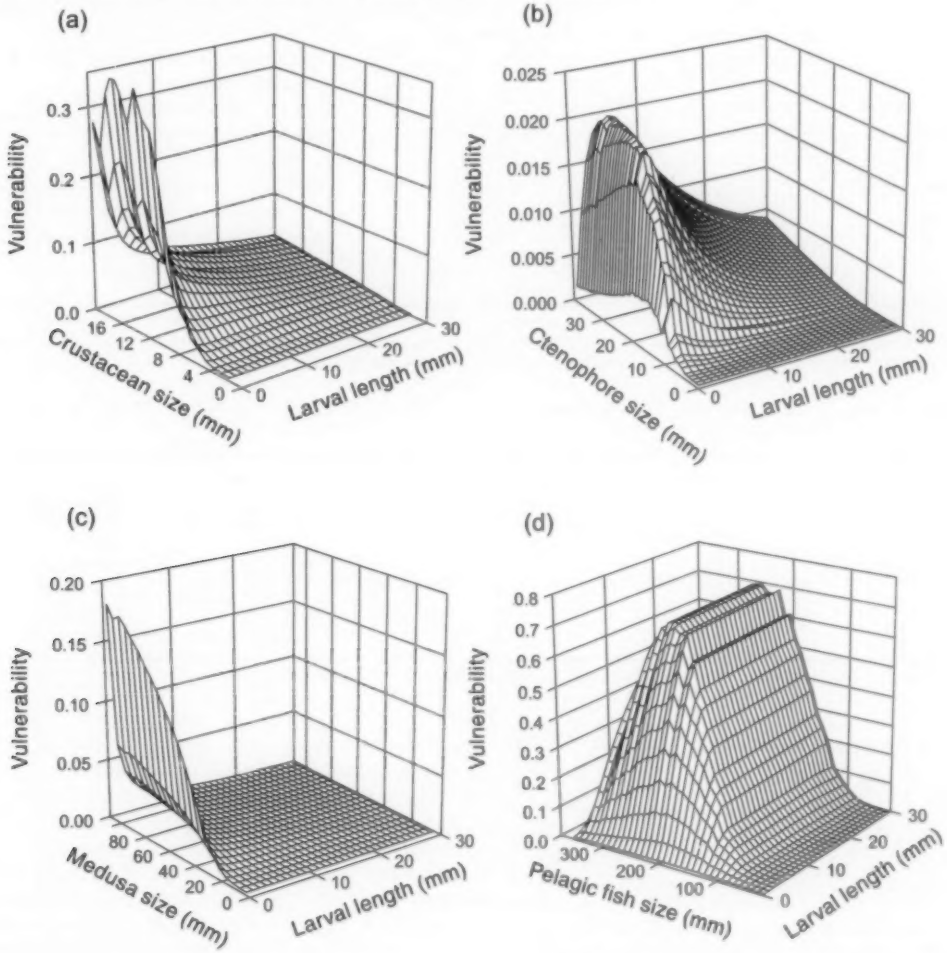
Fig. 1. Vulnerability of fish larvae to predation by (*a*) crustaceans, (*b*) ctenophores, (*c*) medusae, and (*d*) pelagic fishes as functions of both fish larvae length and predator size. Vulnerability was calculated as the product of encounter probability (based on the formulation of Gerritsen and Strickler 1977) and susceptibility (Paradis et al. 1996).



Design of simulations hypotheses

We performed three simulation trials to investigate the three objectives of this study (Table 3). Other simulation studies (Rice et al. 1993*a*, 1993*b*, 1997; Cowan et al. 1996, 1997; Letcher et al. 1996) implicitly assumed that the abundance of predators will only affect the overall survival rate of the cohort and will not interfere with the size-selective mortality of individuals. To evaluate this assumption, we chose five abundance levels for each predator (Table 4). Predator abundance was chosen to yield mortality of the order of 10, 30, 50, 70, and 90% total loss from the initial cohort. We were not concerned whether these abundances were representative of specific field abundance estimates because our main focus was to evaluate how changes in abundances that yield different mortality levels interact with the size-selective mortality of a cohort of fish larvae. For these simulations, we present the characteristics of the cohort of fish larvae consumed by the predators as well as the length frequency distribution of the growth and survival cohorts (Table 3). We also investigated the relationship between size-selective mortality and the instantaneous mortality of a cohort of fish larvae ($Z = (\ln N_0 - \ln N_t)/t$, where N_0 is 10 000 individuals and N_t is the number of survivors after $t = 30$ days). To evaluate the relationship between these two variables, we defined a size-selective mortality index as the absolute maximum difference between the relative cumulative frequency distribution of the growth and survival cohorts. This index is based on the statistic d^+_{max} of the Kolmogorov–Smirnov test for goodness-of-fit for discrete or continuous grouped data (Zar 1984; also see Sokal and Rohlf 1981). We wish to stress that in this study, it is not appropriate to calculate a probability value because we are dealing with true populations and not with samples of a population.

The abundance of predator and prey is often temporally and spatially variable. The presence of pelagic fishes in nursery areas may not coincide temporally with the full developmental period of the early life stages of fishes. We ran a series of simulations in which we restricted predation by pelagic fishes to 5 days (Table 3). In these simulations, the abundance of pelagic fishes was set to $0.0192 \cdot m^{-3}$, which under initial conditions (i.e., no restriction on the timing of predation), 50% of the cohort was consumed in 30 days.

Finally, to evaluate the individual components of vulnerability (i.e., encounter and susceptibility), we manipulated vulnerability by keeping either the encounter rate or the susceptibility probabil-

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Table 3. For the three simulation trials of this study, we give the variable that was manipulated and the output variables that we considered.

Simulation trial	Variable manipulated	Output variables
Predator abundance	A_p (see Table 4)	(*i*) Size of larvae consumed
	Mortality; three levels: M = 10, 50, and 90%	(*ii*) Larvae consumed each day
		(*iii*) Length frequency distribution of survival and growth cohorts
		(*iv*) SMI as a function of Z
Timing of encounters	Time when predation can occur; three levels: t = 1–5, 13–17, and 26–30 days	Length frequency distribution of survival and growth cohorts
Vulnerability components	(*i*) Vulnerability = E; S = 0.5; A_p so that M = 96%	Difference between the size-specific frequency of individuals of the growth and survival cohorts as a function of larval fish length
	(*ii*) Vulnerability = susceptibility; E so that M = 96%	
	(*iii*) Vulnerability = $E \times S$; A_p so that M = 96%	

Note: For the vulnerability components trials, we used 100 000 individuals, and sizes of predators were restricted to single size-classes. A_p, abundance of predators (predators·m^{-3}); M, percentage of the cohort consumed by the predator in 30 days; SMI, size-selective mortality index; Z, instantaneous mortality (%·day^{-1}); t, time (days); S, probability of susceptibility to predation; E, encounter rate (day^{-1}).

Table 4. Abundance of predators that consumed from 10 to 90% of the initial cohort of 10 000 fish larvae in a 30-day simulation period.

	Normal size distribution of predator population (predators·m^{-3})			
Mortality (%)	Crustaceans	Ctenophores	Medusae	Pelagic fishes
10	115	45	16	0.0175
30	180	70	32	0.0184
50	250	145	48	0.0192
70	330	210	75	0.0204
90	480	365	150	0.0225

Note: The size distribution of the predator population was characterized by a normal distribution with parameters given in Table 1.

ity constant and independent of prey and predator sizes (Table 3). Due to the high level of mortality in these simulations (~96% of the initial cohort is consumed in 30 days) and to reduce the stochastic noise in our model, we increased the initial number of fish larvae to 100 000 individuals. This allowed us to better characterize the length frequency distribution of the survival cohort. We reduced the range of predator sizes to a single class (Table 3) to evaluate the effects of both encounter and susceptibility without the confounding effects of variations in predator size. However, we ran other simulations where we relaxed the restriction on predator size by defining the predator population with a range of sizes as in previous simulations (Table 1). We condensed our presentation significantly by presenting the difference between the length frequency distributions at each larval length-class of the growth and survival cohorts (as in Crowder et al. 1994). If this value is positive, it implies that there are relatively more individuals in the growth cohort than in the survival cohort for that given length-class. Therefore, fish larvae of that length-class are most vulnerable to predators.

Results

Abundance of predators

The sizes of fish larvae most vulnerable to predation by crustaceans and pelagic fishes shifted towards smaller sizes as predator abundance increased (Figs. 2*a* and 2*g*, respectively). As predator abundance increased, larval mortality due to these predators occurred earlier during the cohort's life (Figs. 2*b* and 2*h*, respectively). Peak vulnerability to predation by ctenophores and medusae appeared to be independent of predator abundance, as it always occurred early during the cohort's life and for small fish larvae (Figs. 2*c*–2*f*).

The temporal progression of the length frequency distribution of larvae subjected to different types of predation pressure shows that losses occurring early during the development of a cohort appear to have the greatest impact during the late larval phase (Fig. 3). When subject to predation by invertebrates, surviving larvae are always larger than a cohort growing in the absence of predation (Figs. 3*a*–3*c*). The opposite is true with respect to predatory pelagic fishes (Fig. 3*d*). The strongest evidence of size-selective larval mortality occurs for medusae (Fig. 3*c*), which fed heavily on larvae during the first 6 days of the simulations (Fig. 2*f*), after which the larvae quickly grew out of the range of high vulnerability (Fig. 2*e*). It is important to note that the evidence of size-selective mortality only appears when total losses during 30 days exceed 50%, regardless of predator type. Furthermore, by compiling all simulations performed at different predator abundance levels, we find that the index of size-selective mortality increases with instantaneous mortality rates (Fig. 4). In fact, there is almost a one-to-one relationship between these two variables, regardless of predator type.

Temporal variation in predation by pelagic fishes

The strongest evidence of size-selective larval mortality occurred when predation by pelagic fishes was restricted to the first 5 days of the simulation (Fig. 5*a*). There was no difference between the length frequency distributions of the

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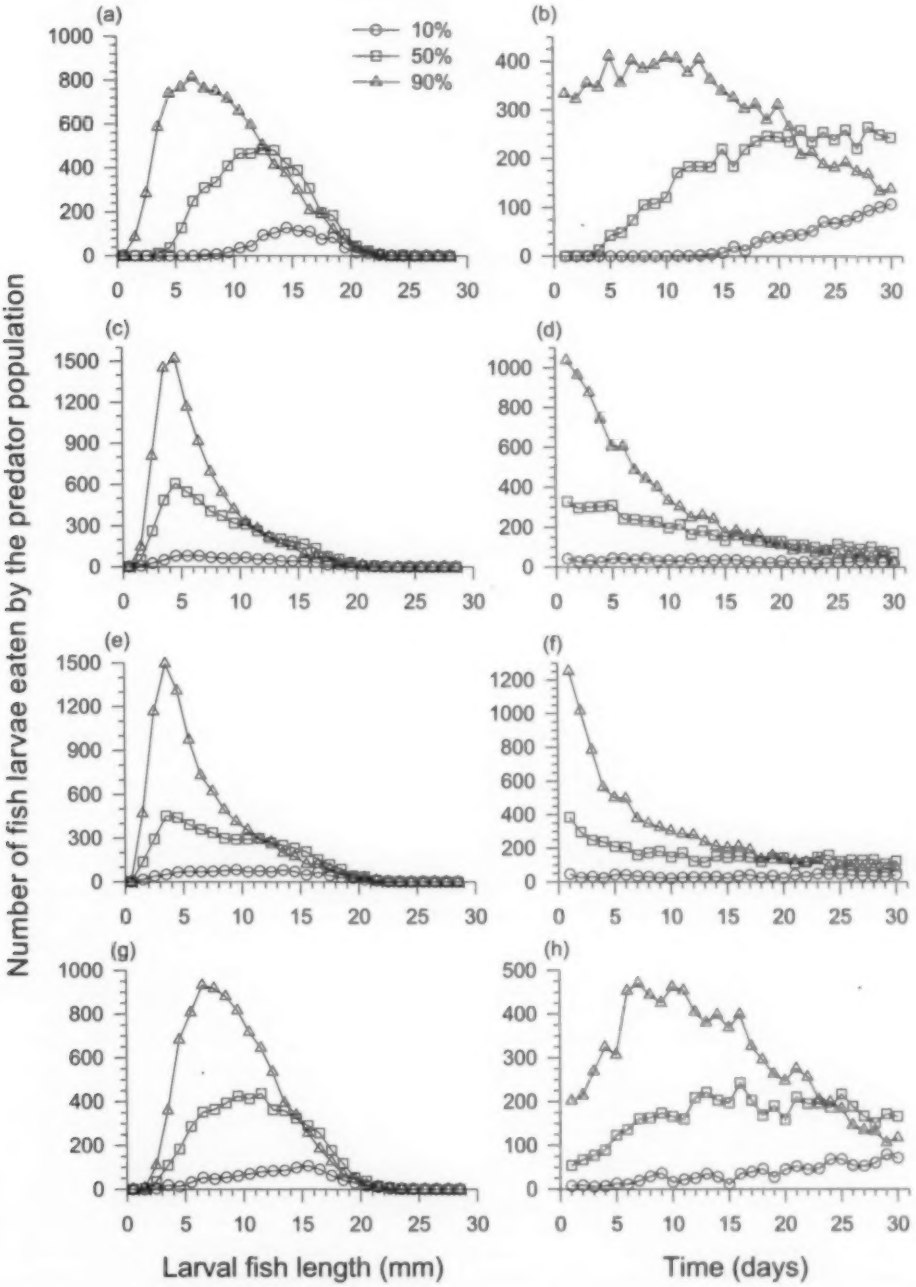
Fig. 2. Amount of fish larvae of specific length consumed by predators (left panels) and amount of fish larvae consumed every day (right panels) by (*a* and *b*) crustaceans, (*c* and *d*) ctenophores, (*e* and *f*) medusae, and (*g* and h) pelagic fishes. Predator abundance was arbitrarily chosen to yield a total mortality of 10, 50, and 90% of the initial cohort of 10 000 fish larvae over 30 days (see values in Table 4).



growth and survival cohorts when predation by pelagic fishes occurred for 5 days either at the middle or at the end of the simulation (Figs. 5*b* and 5*c*). Furthermore, the evidence of size-selective mortality is much stronger if predation by pelagic fishes was restricted to the first 5 days of the simulation (Fig. 5*a*) as compared with the case when predation by pelagic fishes was not restricted and yielded a higher overall mortality rate (Fig. 3*d*). If predation by pelagic fishes

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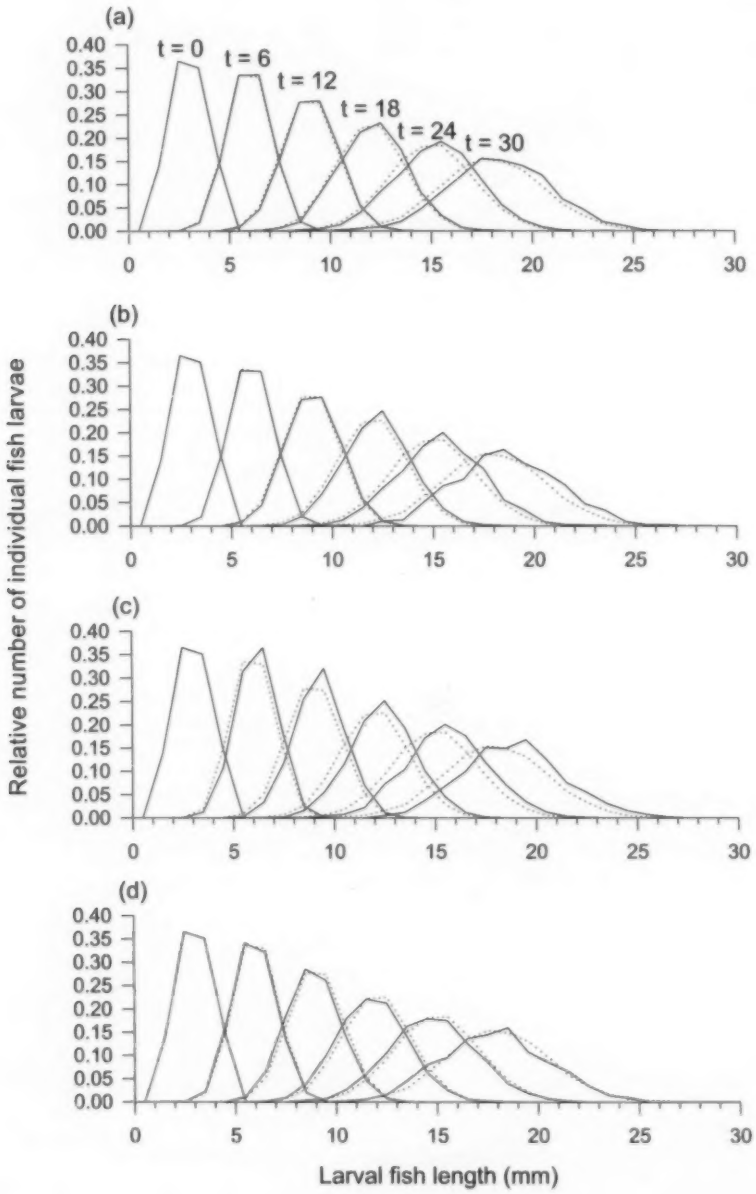
Fig. 3. Length frequency distributions of the growth cohort (no mortality, dotted lines) and survival cohort (with predation mortality, solid lines) at time 0 and every sixth day until the end of the simulation run (day 30). The survival cohort was subjected to predation by (*a*) crustaceans, (*b*) ctenophores, (*c*) medusae, and (*d*) pelagic fishes. Abundance of predators was arbitrarily chosen to yield a mortality of 90% of the initial cohort of 10 000 individuals in 30 days (see values in Table 4).



is restricted to the first 5 days of the simulation, fish larvae present at that time measure between 2 and 9 mm in length, a size range that is most vulnerable to predation by pelagic fishes (Figs. 2*g* and 2*h*).

Disentangling the effects of encounter and susceptibility

If vulnerability of fish larvae is entirely dependent on encounter, mortality increases with increasing larval length and the survival cohort is smaller in length than the growth cohort. Individuals 18 mm or longer are relatively more numerous in the growth cohort than in the survival cohort, indicating that relative mortality (based on encounter) of these individuals is higher (Fig. 6). The evidence of size selection (expressed as the percent difference between the

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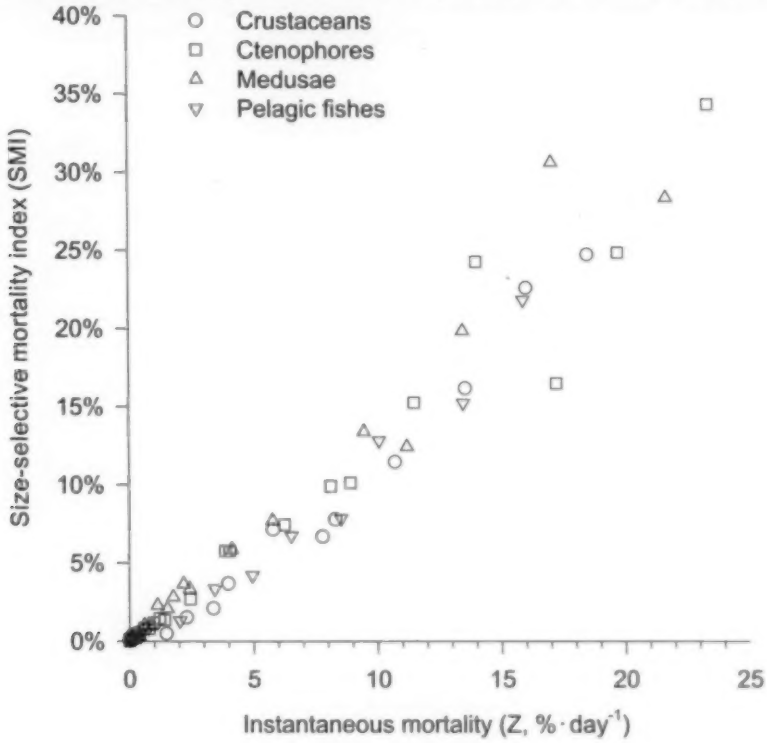
Fig. 4. SMI measured as the maximum difference between the cumulative length frequency distributions of the growth cohort (no mortality) and the survival cohort (with predation) at the end of the 30-day simulation as a function of the instantaneous mortality rate.



length frequency distributions of the growth and survival cohorts) decreases slightly with increasing invertebrate size (Figs. 6*a*–6*c*). There is no more than a 2% size-specific difference between the growth and survival cohorts when larvae are faced with predation by pelagic fishes (Fig. 6*d*).

If vulnerability is entirely dependent on susceptibility, mortality by invertebrates decreases with increasing larval length. The survival cohort is slightly larger than the growth cohort because fish larvae less than 18 mm are most vulnerable to predation by invertebrates and by small size-classes of pelagic fishes (Fig. 7). Size-selective mortality (based on susceptibility) is similar for all three invertebrate predators and is independent of crustacean and medusa size-classes (Figs. 7*a* and 7*c*). However, it varies considerably with the size-classes of pelagic fishes (Fig. 7*d*). Small fish larvae (less than 18 mm) have a higher susceptibility to predation by small pelagic fishes (25 and 45 mm) whereas individuals larger than 18 mm have a higher susceptibility to predation by the longest pelagic fishes (355 mm). There is no evidence of size-selective mortality (based on susceptibility) of fish larvae from predation by pelagic fishes measuring 115 mm.

Size-dependent encounter tends to favour smaller fish larvae (Fig. 6) whereas size-dependent susceptibility tends to favour larger fish larvae (Fig. 7). When vulnerability is a function of both of these size-dependent relationships, we find that the evidence of size-selective mortality due to predation by crustaceans decreased from ±5 to ±3% (Figs. 6*a*, 7*a*, and 8*a*) whereas it increased from ±4 to ±5 to more than ±6% for ctenophores (Figs. 6*b*, 7*b*, and 8*b*). The evidence of size-selective mortality due to predation by medusae (Figs. 7*c* and 8*c*) or pelagic fishes (Figs. 7*d* and 8*d*) remained approximately as size-dependent susceptibility would predict (i.e., ±4%). These results suggest that susceptibility is the driving force of vulnerability when 96% of the initial cohort is consumed in 30 days. When we compare these results with simulations where the predator population is characterized by a wider range of sizes, we find that the evidence of size-selective predation by crustaceans or ctenophores decreased slightly (Figs. 8*a* and 8*b*). For medusae, there is very little effect of the range in medusae sizes on the size-selective mortality of fish larvae (Fig. 8*c*). There was little evidence of size-selective predation by pelagic fishes as the range of predator size increased (Fig. 8*d*).

Discussion

Our simulation results clearly show that predation pressure must be substantial in order to show any evidence of size-selective losses from larval fish populations. The evidence of size-selective losses appeared to be greatest for invertebrate predators relative to vertebrate predators when predation pressure was exerted throughout the simulations. The greater effect of invertebrate predators was partly because the timing of losses during the life of larvae was concentrated early in the model runs. This was made even more apparent when predation by pelagic fishes was restricted to

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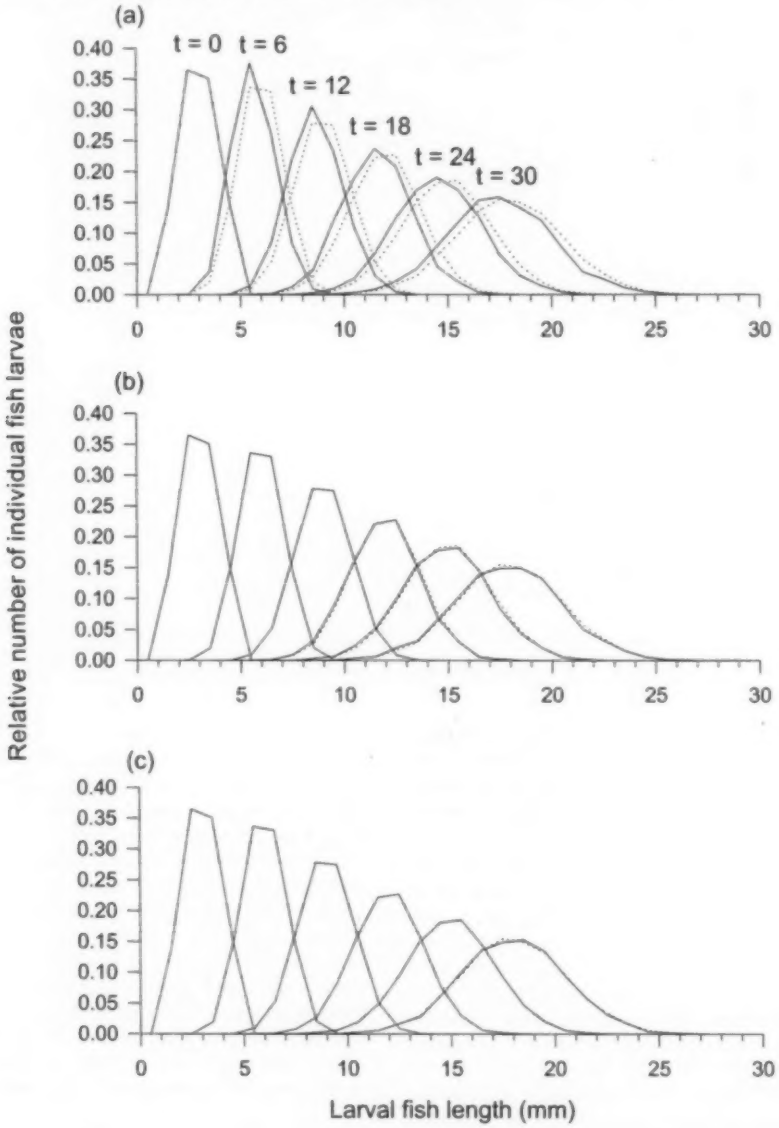
Fig. 5. Length frequency distributions of the growth cohort (no mortality, dotted lines) and survival cohort (with predation mortality, solid lines) at time 0 and every sixth day until the end of the simulation run (day 30). Predation by pelagic fishes was restricted to 5 days (*a*) early in the simulation period (days 1–5), (*b*) in the middle of the simulation period (days 13–17), or (*c*) late in the simulation period (days 26–30). Abundance of pelagic fishes was $0.0192 \cdot m^{-3}$.



short 5-day periods, as only predation during the earliest part of the simulation yielded any evidence of size selection after a 30-day simulation. However, throughout the simulated conditions, evidence of size selection was relatively weak. We demonstrated that this occurred because the general model of size-dependent susceptibility (Paradis et al. 1996) counteracted Gerritsen and Strickler's (1977) widely used model of size-dependent encounter rates.

We found that size-selective mortality was stronger when predation by pelagic fishes was restricted to the first 5 days of the simulation, in contrast with predation that was restricted later during the simulation period. This pattern of size-selective mortality is also much stronger than that of a nonrestricted period of predation. The difference between the length frequency distributions of the survival cohort and the growing cohort is enhanced with time through the action of growth because almost all of the larger fish larvae were eaten during the first 5 days of their developmental period. If predation by pelagic fishes is not restricted to the early period of larval development, the overall mortality of fish larvae is spread out over the simulation period, reducing the evidence of size-selective mortality. We can reasonably as-

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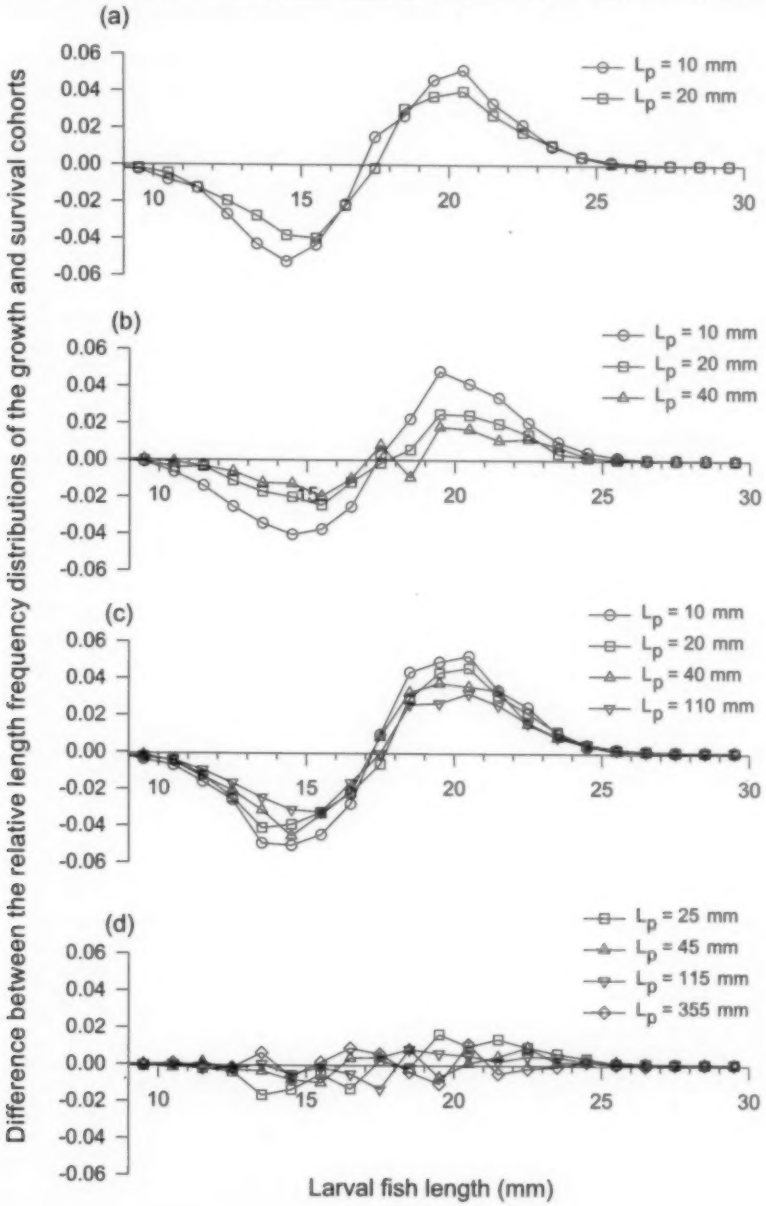
Fig. 6. Difference between the length frequency distributions of the growth cohort (no mortality) and survival cohort (with predation) at the end of the 30-day simulation. Vulnerability due to predation by (*a*) crustaceans, (*b*) ctenophores, (*c*) medusae, and (*d*) pelagic fishes is dependent only on encounter rate because susceptibility is constant. For each simulation, the predator population was composed of a single size-class (L_p). Abundance of predators was set to yield a mortality of 96% of the initial cohort of 100 000 individuals.



sume that this brief temporal overlap during the early part of the larval period may occur in the field because of the ample evidence of temporal overlap between pelagic fishes and fish eggs and larvae (Hunter and Kimbrell 1980; Santander et al. 1983; Daan et al. 1985; Cowan and Houde 1993).

In other individual-based simulation models (e.g., Rice et al. 1993*a*, 1993*b*, 1997; Cowan et al. 1996, 1997; Letcher et al. 1996), abundance of predators was selected to yield significant predation rates comparable with field estimates of mortality rates of fish larvae. Furthermore, the predator abundance modeled was comparable with field abundance estimates of predators. However, very little is known about the pattern of size-selective predation mortality given variations in predator abundance and ultimately the overall mortality rate of the cohort of fish larvae. If the length distribution of surviving fish larvae varies with variation of

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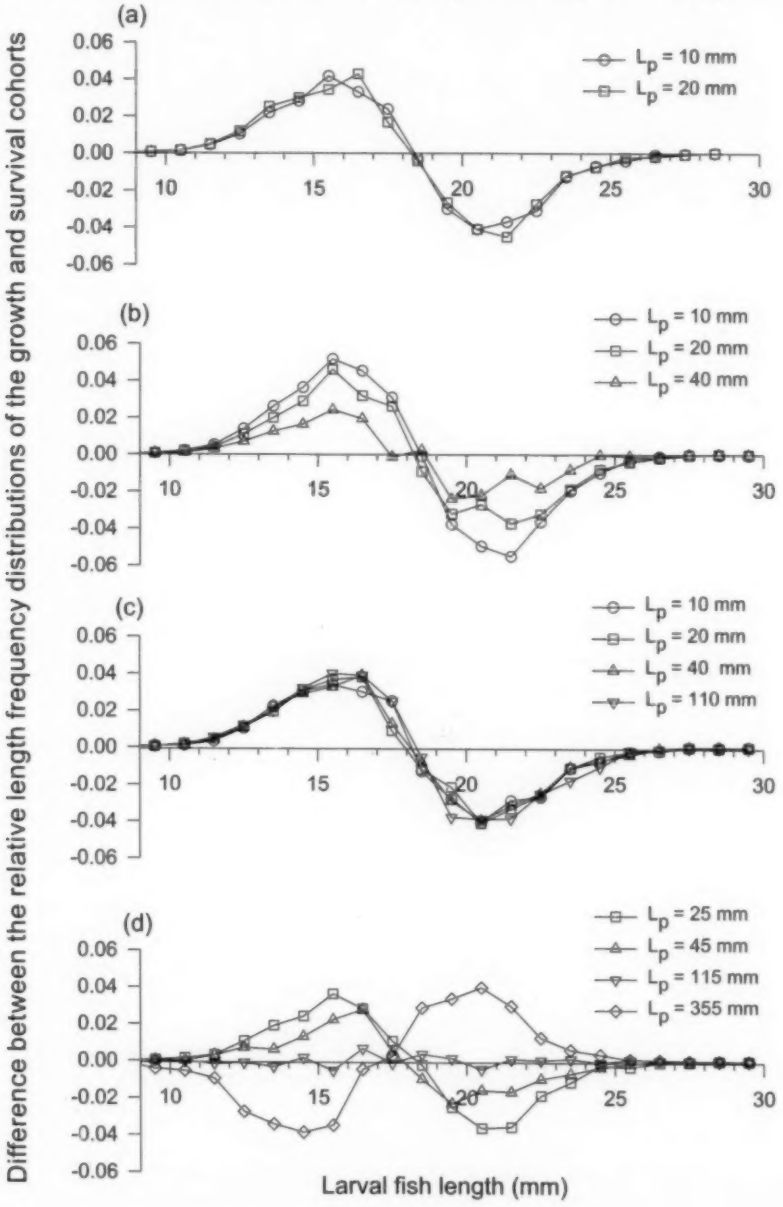
Fig. 7. Difference between the length frequency distributions of the growth cohort (no mortality) and survival cohort (with predation) at the end of the 30-day simulation. Vulnerability due to predation by (*a*) crustaceans, (*b*) ctenophores, (*c*) medusae, and (*d*) pelagic fishes is dependent only on susceptibility because encounter rate is constant and set to yield a mortality of 96% of the initial cohort of 100 000 individuals. For each simulation, the predator population was composed of a single size-class (L_p).



predator abundance, it could become particularly problematic if one compares different fish larvae characteristics at different predator abundances (e.g., Cowan et al. 1996). We investigated the characteristics of fish larvae consumed under different levels of predator abundance and found that the time when most mortality occurs changes the length distribution of fish larvae consumed by predators and consequently the length distribution of survivors. In order to see responses of the length frequency distribution of surviving fish larvae, the impact of predation must occur during a restricted period, either by high selection on a narrow range of larval lengths or by high mortality restricted to a few days. In the field, invertebrate predators do not feed on such a restricted size range of larvae or for a short time period.

Characteristics of the predator size distribution have a strong influence in determining the sizes (Rice et al. 1993*b*;

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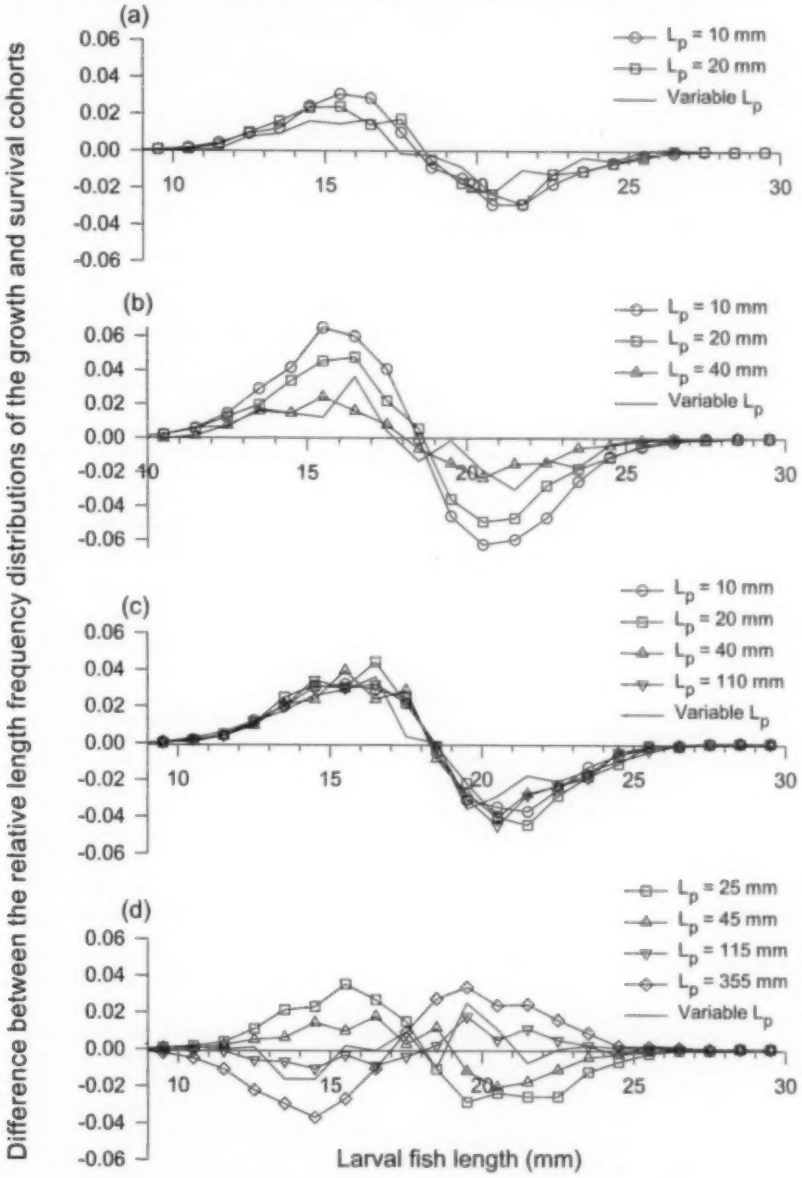
Fig. 8. Difference between the length frequency distributions of the growth cohort (no mortality) and survival cohort (with predation) at the end of the 30-day simulation. Vulnerability due to predation by (*a*) crustaceans, (*b*) ctenophores, (*c*) medusae, and (*d*) pelagic fishes is dependent on encounter and susceptibility but independent of predator size because the predator population was composed of a single size-class (L_p). However, for the four predator types, we relaxed the restriction of single predator size-class (variable L_p). Abundance of predators was set to yield a mortality of 96% of the initial cohort of 100 000 individuals.



Letcher et al. 1996) or growth rates (Rice et al. 1993*a*) of surviving fish larvae. We found that the length distribution of surviving fish larvae changes with different single size-classes of pelagic fishes. We also found that the difference between the length frequency distributions of the growth and the survival cohorts decreases only slightly when the predator population is characterized by a wider range of sizes. This result contradicts Rice et al.'s (1993*a*, 1993*b*) suggestion that a wider range of sizes would significantly decrease the evidence of size-selective mortality of fish larvae. We suggest that this result is not due to different sizes of predators consuming different sizes of fish larvae but mostly due to the different timing of high vulnerability caused by the different predator sizes. High susceptibility occurs when a fish larva measures 10% of the length of a predator (Paradis et al. 1996). If we compare simulation trials of different

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predator sizes, the time at which the cohort of fish larvae is highly susceptible to predation will differ between trials because fish larvae will measure 10% the length of the predator at different times during the simulation.

We demonstrated clearly that the encounter and susceptibility formulations (used in our model) counteract each other. Cowan et al. (1996) used the same encounter model (Gerritsen and Strickler 1977), but their results showed much greater evidence of size-selective mortality. The difference between these results is likely due to the choice of susceptibility function used. Cowan et al. (1996) used a susceptibility function based on their own laboratory work, derived to answer specific questions within their modeled system. We used empirically derived predation rates corrected for laboratory conditions (Paradis et al. 1996). We argue, as did Rice et al. (1997), that an empirical derivation of laboratory estimates of predation rates, based on multispecies prey and predator, is probably a better generalization of the susceptibility patterns of larval fishes. Furthermore, the parameters of such general functions are not specifically derived under a single set of experimental conditions. There is no reason to believe that one particular set of experimental conditions is likely to yield more realistic estimates of susceptibility. In addition, when prey and predators are studied in laboratory containers or enclosures, they are effectively placed in contact with each other, in contrast with field situations where spatial overlap may vary (Appendix) (Frank and Leggett 1982, 1985). When vulnerability is a function of both size-dependent encounter rate and size-dependent susceptibility, and the predator population is characterized by a wide range of sizes, we predict that the highest difference between the length frequency distributions of survivors and of growing fish larvae without predation will be less than 10%.

We seldom found notable differences in sizes between a cohort of fish larvae surviving different predation pressures and a cohort of fish larvae growing without mortality. Size-selective mortality due to predation of fish larvae may therefore be difficult to evaluate in the field (also see Miller 1997). We were able to demonstrate that this lack of size-selective mortality is partly due to the counteracting components of vulnerability. We also demonstrated that when the range of predator sizes was restricted to a single size-class, there could be a reversal in the direction of size-selective survival of the cohort of fish larvae. Finally, we demonstrated a direct relationship between size-selective predation mortality (defined as the maximum difference between cumulative length frequency distributions of the cohort of survivors and the cohort of growing fish larvae) and the instantaneous mortality rate of the cohort (Fig. 8). This relationship clearly demonstrates that size-selective predation mortality will be detected only in cases where mortality rates are high (greater than 10%·day^{-1}) or in cases where a population of pelagic fishes consumes a significant amount of fish larvae during only a brief period during the early development of larvae. Despite problems relating to the tremendous variability in the temporal and spatial distribution of fish larvae and their predators, we believe that with appropriate techniques, it is possible to estimate size-specific mortality rates due to predation in the field (also see Miller 1997). However, caution should be exercised during the investigation of the individual components of vulnerability.

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References

Bailey, K.M., and Batty, R.S. 1983. A laboratory study of predation by *Aurelia aurita* on larval herring (*Clupea harengus*): experimental observations compared with model predictions. Mar. Biol. **72**: 295–301.

Bailey, K.M., and Houde, E.D. 1989. Predation on eggs and larvae of marine fishes and the recruitment problem. Adv. Mar. Biol. **25**: 1–83.

Cowan, J.H., Jr., and Houde, E.D. 1992. Size-dependent predation on marine fish larvae by ctenophores, schyphomedusae, and planktivorous fish. Fish. Oceanogr. **1**: 113–126.

Cowan, J.H., Jr., and Houde, E.D. 1993. Relative predation potentials of scyphomedusae, ctenophores and planktivorous fish on ichthyoplankton in Chesapeake Bay. Mar. Ecol. Prog. Ser. **95**: 55–65.

Cowan, J.H., Jr., Houde, E.D., and Rose, K.A. 1996. Size-dependent vulnerability of marine fish larvae to predation: an individual-based numerical experiment. ICES J. Mar. Sci. **53**: 23–37.

Cowan, J.H., Jr., Rose, K.A., and Houde, E.D. 1997. Size-based foraging success and vulnerability to predation: selection of survivors in individual-based models of larval fish populations. *In* Early life history and recruitment in fish populations. *Edited by* R.C. Chambers and E.A. Trippel. Chapman and Hall, London, U.K. pp. 357–386.

Crowder, L.B., Wright, R.A., Rose, K.A., Martin, T.H., and Rice, J.A. 1994. Direct and indirect effects of southern flounder predation on a spot population: experimental and model analyses. *In* Theory and application in fish feeding ecology. *Edited by* D.J. Stouder, K.L. Fresh, and R.J. Feller. University of South Carolina Press, Columbia, S.C. pp. 61–77.

Daan, N., Rijnsdorp, A.D., and van Overbeeke, G.R. 1985. Predation by North Sea herring *Clupea harengus* on eggs of plaice *Pleuronectes platessa* on cod *Gadus morhua*. Trans. Am. Fish. Soc. **114**: 499–506.

Devroye, L. 1986. Non-uniform random variate generation. Springer-Verlag, New York.

Frank, K.T., and Leggett, W.C. 1982. Coastal water mass replacement: its effect on zooplankton dynamics and the predator–prey complex associated with larval capelin (*Mallotus villosus*). Can. J. Fish. Aquat. Sci. **39**: 991–1003.

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Frank, K.T., and Leggett, W.C. 1985. Reciprocal oscillations in densities of larval fish and potential predators: a reflection of present or past predation? Can. J. Fish. Aquat. Sci. **42**: 1841–1849.

Gerritsen, J., and Strickler, J.R. 1977. Encounter probabilities and community structure in zooplankton: a mathematical model. J. Fish. Res. Board Can. **34**: 73–82.

Greene, C.H. 1986. Patterns of prey selection: implications of predator foraging tactics. Am. Nat. **128**: 824–839.

Houde, E.D. 1987. Fish early life dynamics and recruitment variability. Am. Fish. Soc. Symp. **2**: 17–29.

Hunter, J.R., and Kimbrell, C.A. 1980. Egg cannibalism in the northern anchovy, *Engraulis mordax*. Fish. Bull. U.S. **78**: 811–816.

Letcher, B.H., Rice, J.A., Crowder, L.B., and Rose, K.A. 1996. Variability in survival of larval fish: disentangling components with a generalised individual-based model. Can. J. Fish. Aquat. Sci. **53**: 787–801.

Lillelund, K., and Lasker, R. 1971. Laboratory studies of predation by marine copepods on fish larvae. Fish. Bull. U.S. **69**: 655–667.

Miller, T.J. 1997. The use of field studies to investigate selective processes in fish early life history. *In* Early life history and recruitment in fish populations. *Edited by* R.C. Chambers and E.A. Trippel. Chapman and Hall, London, U.K. pp. 197–223.

Paradis, A.R., Pepin, P., and Brown, J.A. 1996. Vulnerability of fish eggs and larvae: an empirical model of predation and the relative size of prey to predators. Can. J. Fish. Aquat. Sci. **53**: 1226–1235.

Rice, J.A., Miller, T.J., Rose, K.A., Crowder, L.B., Marshall, E.A., Trebitz, A.S., and DeAngelis, D.L. 1993*a*. Growth rate variation and larval survival: inferences from an individual-based size-dependent predation model. Can. J. Fish. Aquat. Sci. **50**: 133–142.

Rice, J.A., Crowder, L.B., and Rose, K.A. 1993*b*. Interactions between size-structured predator and prey populations: experimental test and model comparison. Trans. Am. Fish. Soc. **122**: 481–491.

Rice, J.A., Crowder, L.B., Marshall, E.A. 1997. Predation on juvenile fishes: dynamic interactions between size-structured predators and prey. *In* Early life history and recruitment in fish populations. *Edited by* R.C. Chambers and E.A. Trippel. Chapman and Hall, London, U.K. pp. 333–356.

Santander, H., Alheir, J., MacCall, A.D., and Alamo, A. 1983. Egg mortality of the Peruvian anchovy (*Engraulis ringens*) caused by cannibalism and predation by sardines (*Sardinops sagax*). *In* Proceedings of the Expert Consultation to Examine Changes in Abundance and Species Composition of Neritic Fish Resources. *Edited by* G.D. Sharp and J. Csirke. FAO Fish. Rep. **291**: 1011–1025.

Sokal, S.R., and Rohlf, F.J. 1981. Biometry. Freeman and Company, New York.

von Westernhagen, H., Rosenthal, H., Kerr, S., and Furstenberg, G. 1979. Factors influencing predation of *Hyperoche medusarum* (Hyperiida: Amphipoda) on larvae of the Pacific herring *Clupea harengus pallasi*. Mar. Biol. **51**: 195–201.

Ware, D.M. 1975. Growth, metabolism and optimal swimming speed of pelagic fish. J. Fish. Res. Board Can. **32**: 33–41.

Werner, E.E., and Gilliam, J.F. 1984. The ontogenetic niche and species interactions in size-structured populations. Annu. Rev. Ecol. Syst. **15**: 393–425.

Zar, J.H. 1984. Biostatistical analysis. Prentice-Hall, Englewood Cliffs, N.J.

Appendix

Paradis et al. (1996) have derived empirical predation rates based on multispecific experimental laboratory work. Can we assume that these laboratory-derived and empirically derived predation rates are susceptibility functions in field analyses? Under experimental conditions used to date to quantify laboratory predation rates, can we assume that prey and predator are enclosed in the same body of water? In other words, encounter rates, according to Gerritsen and Strickler's (1977) formulation, must be ≥1.0. This would imply that predation rates measured from laboratory experiments are actually susceptibility functions on a broader temporal and spatial scale (i.e., in the ocean). The averaged experimental conditions that may affect predation rates via the encounter process were as follows:

Variable	Container volume (m^3)	Duration of experiment (h)	N_p (no.)	L_p (mm)	L_f (mm)
Crustaceans	0.00755	20.8	3.9	9.95	7.23
Ctenophores	0.0172	5.78	1.4	18.8	3.2
Fishes	3.55	7.48	2.7	113.1	12.9
Medusae	1.7065	20.67	4.8	33.8	8.35

where N_p is the number of predators used on average in experimental study of predation.

According to Gerritsen and Strickler's (1977) model of encounter (eq. 1 in the text) and if we substitute the averaged value from the table above, we find that the encounter rates during the mean duration of laboratory experiments were $E_{crustaceans} = 20.0$ encounters, $E_{ctenophores} = 3.0$ encounters, $E_{fishes} = 34.9$ encounters, and $E_{medusae} = 2.0$ encounters.

We can safely assume that under averaged experimental conditions, fish larvae and their predators are enclosed in the same body of water because encounter rates (according to Gerritsen and Strickler 1977) are greater than 1. This implies that the measured predation rates of averaged experimental conditions are actually measures of susceptibility to predation. If we wish to use these predation rates at greater spatial and temporal scales such as those in the field, we should assume that these predation rate estimates are in fact estimates of the function of susceptibility.

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Is it possible to predict habitat use by spawning salmonids? A test using California golden trout (*Oncorhynchus mykiss aguabonita*)

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Abstract: It is widely believed that stream salmonids select spawning sites based on water depth, water velocity, and substrate size. Attempts to predict spawning locations using these habitat features have met with little success, however. In this study, we used nonparametric logistic regression to determine what habitat features were associated with the locations chosen by spawning California golden trout (*Oncorhynchus mykiss aguabonita*). From this nonparametric model, we developed a parametric model that incorporated the habitat features most strongly associated with spawning sites and used this model to calculate the probability of use by spawning golden trout for specific stream locations. The overall nonparametric model was highly significant and explained 62% of the variation in spawning location. Four of the eight habitat variables, substrate size, water depth, water velocity, and stream width, had highly significant effects and alone explained 59% of the variation in spawning location. The results of a cross-validation procedure indicated that the parametric model generally provided a good fit to the data. These results indicate that location-specific probabilities of use were predictable based on easily measured habitat characteristics and that nonparametric regression, an approach still rarely used in ecological studies, may have considerable utility in the development of fish–habitat models. Given the escalating pace at which fish habitats are being altered, such models are increasingly important in predicting the effects of these alterations on populations.

Résumé : Il semble bien établi que les salmonidés des cours d'eau choisissent leur frayère selon la profondeur de l'eau, la vitesse du courant et la taille du substrat. Cependant, des essais de prédiction de l'emplacement des frayères en utilisant ces caractéristiques de l'habitat ont échoué. La présente étude utilise la régression logistique non paramétrique pour déterminer les caractéristiques de l'habitat associées aux frayères choisies par la truite dorée (*Oncorhynchus mykiss aguabonita*) de Californie. À l'aide du modèle non paramétrique, nous avons développé un modèle paramétrique qui tient compte des caractéristiques de l'habitat les plus étroitement associées aux lieux de fraye et nous l'avons utilisé pour calculer la probabilité d'utilisation par les truites dorées pour la fraye d'endroits spécifiques dans les cours d'eau. En général, le modèle non paramétrique était très représentatif et expliquait 62 % des variations dans le choix des frayères. Quatre des huit variables (taille du substrat, profondeur de l'eau, vitesse du courant et largeur du cours d'eau) avaient des effets extrêmement importants et, à elles seules, expliquaient 59 % des variations dans le choix des frayères. Les résultats d'une procédure de contrevalidation ont montré que le modèle paramétrique permettait d'ajuster les données de façon satisfaisante. Ces résultats ont aussi fait ressortir que la probabilité d'utilisation d'un lieu spécifique pouvait être prédite en fonction de caractéristiques de l'habitat facilement mesurables et que la régression non paramétrique, approche encore peu utilisée dans les études écologiques, pouvait être très utile pour développer des modèles d'habitat du poisson. Le rythme accéléré auquel les habitats du poisson sont modifiés rend indispensables des modèles qui prévoient les effets de ces modifications sur les populations.

[Traduit par la Rédaction]

Introduction

The effects of habitat conditions on stream fish populations are frequently evaluated using microhabitat models (e.g., Instream Flow Incremental Methodology (IFIM) and associated Physical Habitat Simulation (PHABSIM) System; Bovee 1978; Reiser et al. 1989). These models are based on the assumption that fish selectively occupy habitats as a function of their preferences for habitat characteristics such as water depth, water velocity, and substrate type (Stalnaker 1979); if true, then by knowing the distributions of these habitat features and the preferences of fish for these features, the distribution of fish within a stream should be predictable (Beecher et al. 1993). While several studies based on fish

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distributions measured during the nonreproductive season have provided support for this central tenet (Beecher et al. 1993; Nehring and Anderson 1993), there have been very few attempts to predict locations chosen by spawning fish. In the only such published study that we have found, Shirvell (1989) reported a very poor correspondence between the locations predicted by measurements of water depth, water velocity, and substrate size as containing suitable spawning habitat and the locations actually used by spawning fish. This poor model performance has led some to suggest that stream salmonids select spawning locations based on habitat characteristics other than or in addition to depth, velocity, and substrate (Shirvell 1989; Beecher et al. 1993). Indeed, several studies have indicated the importance of groundwater seepage (Witzel and MacCrimmon 1983; Lorenz and Eiler 1989), river bottom topography (Shirvell 1989), and proximity to streambanks (Stefferud 1993) in influencing spawning site selection. Despite the uncertainty over the relative importance of these habitat characteristics in influencing spawning locations, important stream management decisions such as instream flow recommendations are routinely made based in part on spawning habitat availability as predicted solely by water depth, water velocity, and substrate size (e.g., California Department of Fish and Game 1996).

The goal of the present study was to first determine what habitat characteristics influence the locations chosen by spawning California golden trout (*Oncorhynchus mykiss aguabonita*) and then test the hypothesis that the probability of individual stream locations being used by spawning fish is predictable based on these fish–habitat relationships. To test this hypothesis, we measured channel and microhabitat characteristics in a stream located in the southern Sierra Nevada, California, and then used nonparametric logistic regression to identify those stream features associated with the presence of golden trout redds. After identifying which of the measured variables were most closely associated with spawning locations, we developed a parametric model based on these variables to predict the probability of use by spawning golden trout. We then compared these predicted probabilities of use with the actual locations used by spawning golden trout.

Materials and methods

Study site

Mulkey Creek is a tributary to the upper South Fork Kern River, a watershed located at the southern end of the Sierra Nevada, Inyo National Forest, California (118°15′N, 36°22′W). The portion of Mulkey Creek used in this study was located at the lower end of Mulkey Meadow at an elevation of about 2850 m. A map and detailed description of the study area are provided in Knapp and Vredenburg (1996) and Knapp et al. (1998). The California golden trout is the only fish species present in Mulkey Creek.

Two sections of Mulkey Creek were used in this study and were chosen because of their similarly low stream gradients, gradients typical of the meadow streams in the South Fork Kern River drainage in which the golden trout is native. Each of these study sections was about 750 m long and, based on the frequent observation of spawning activity in these sections during previous years (Knapp and Vredenburg 1996), contained abundant spawning habitat. These two study sections were separated by a 1.5-km stream section characterized by a substantially higher stream gradient. Because high stream gradients are unusual in the meadow streams of the South Fork Kern River drainage, we did not use this section in our study. In the two study sections, the stream channel alternated between narrow, deep reaches (E channels; Rosgen 1994) and wide, shallow reaches (C channels; Rosgen 1994). Based on these channel types, we divided the stream within the two study sections into 11 distinct reaches (six C channel reaches and five E channel reaches). Reaches ranged in length from 45 to 130 m. We made measurements of channel morphology and stream microhabitat characteristics in all 11 reaches and used these data to develop the logistic regression model.

Data collection

We measured characteristics of redds and stream channels immediately after the 1996 spawning period to ensure that measurements reflected those encountered by spawning trout (end of spawning: June 5; habitat measurements: June 6–12; Knapp et al. 1998). All channel and instream characteristics were measured along transects spaced 2.5 m apart and arranged perpendicular to stream flow (number of transects per reach = 20–46). At each transect, we measured stream width, bank angle, and streambank vegetation cover. Stream width (excluding islands) was measured to the nearest 5 cm with a meter tape. We measured the angle of both banks to the nearest 5° using a clinometer on a 1.5-m rod placed against the bank slope (Platts et al. 1983); lower angles indicate more bank overhang. Bank vegetation cover was estimated visually on both banks inside a 0.1-m^2 area extending from the stream edge to 50 cm away from the stream and 10 cm upstream and downstream of the transect.

We measured water depth (to the nearest 2 cm), water velocity, and substrate size at five equally spaced points along each transect. Water velocity was measured with an electromagnetic current meter to the nearest 1 $cm \cdot s^{-1}$ at 60% of the water depth, and each measurement represented a 10-s average. We visually estimated the average substrate size within a 10 × 10 cm area centered at each point. Visual estimates of average substrate size were highly correlated with the actual geometric mean diameter calculated from core samples ($n = 20$, $R^2 = 0.93$, $P < 0.0001$; Knapp et al. 1998).

Stream channel maps for the study reaches were made by tracing the stream margins from high-resolution aerial photographs of the study area. Locations of transects, microhabitat measurement points, and redds were drawn onto these maps (Fig. 1) and the maps were then used to divide the study reaches into a series of "cells." A cell was a roughly rectangular portion of the stream surface whose dimensions were determined by the spacing of the stream channel transects and the five microhabitat measurement points across each transect (Fig. 1). Each cell had at its center one of the microhabitat measurement points and extended on average 1.25 m upstream (i.e., half way to the next transect) and 1.25 m downstream and was one fifth as wide as the stream at that transect (Fig. 1). Water depth, velocity, and substrate size from each microhabitat measurement point were assigned to the associated cell. A total of 1765 cells were used in this study, with individual study reaches containing 100–230 cells. We considered a cell as having been utilized by spawning fish if it contained any portion of a redd. The position of each redd relative to the streambank was estimated as the distance from the center of the cell containing the redd to the nearest streambank.

Statistical analyses

Habitat description

Differences in habitat characteristics associated with cells used and not used by spawning golden trout were analyzed using the nonparametric Kruskal–Wallis one-way analysis of variance. We used a nonparametric test because habitat variables were typically

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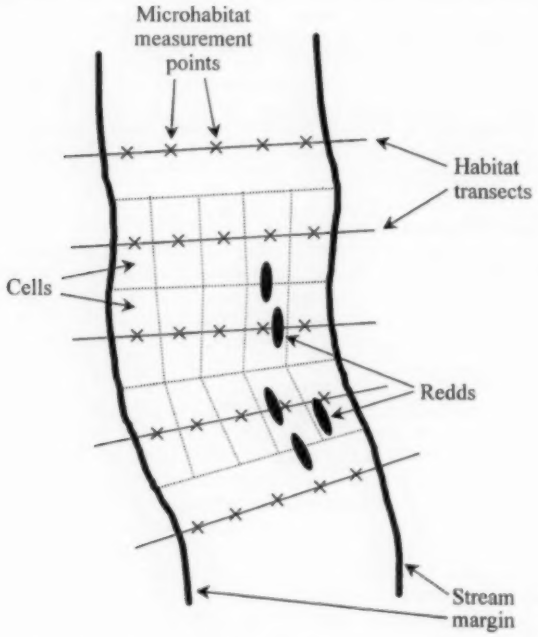
Fig. 1. Portion of a stream channel map showing how cells were created from habitat transects and microhabitat measurement points.



not normally distributed and had unequal variances, and normality and variance equivalency generally could not be achieved using standard transformations.

Model development

We used nonparametric logistic regression to describe the relationship between the independent variables (channel and microhabitat characteristics) and the probability of finding redds at a given location (probability of response). Nonparametric logistic regression models are a subclass of generalized additive models (GAMs) and as such relax the assumptions of ordinary linear regression in two ways: (*i*) the distribution of the dependent variable need not be symmetric or homoscedastic and (*ii*) the relationship between the dependent and independent variables need not be linear. Relaxation of the assumption of a linear relationship between the dependent and independent variables is accomplished using a spline smoothing function (e.g., loess) to determine the fitted model that best fits the independent variables (Cleveland and Devlin 1988; Efron and Tibshirani 1991). Nonparametric logistic regression approaches are likely to be of considerable utility in studies of habitat selection by fishes, since numerous response-curve shapes are typically plausible but the actual shape will likely be unknown prior to analysis. Researchers have only recently begun to use nonparametric regression models in ecological studies, however (e.g., Wiser et al. 1998).

Because the shapes of the curves describing the probability of response in our study were unknown prior to analysis and we had no reason to believe that the relationships were linear, nonparametric logistic regression was ideally suited for analyzing the effect of habitat characteristics on the probability of finding redds at a given location. Specifically, if p_i is the probability of finding redds at location i, then

$$(1) \qquad p_i = \frac{e^{\theta_i}}{1 + e^{\theta_i}}$$

where the linear predictor (i.e., logit line) θ_i is some function of the independent variables. The relationship we used for θ_i was as follows:

$$(2) \quad \theta_i = \beta_0 + g_1(\text{water depth}_i) + g_2(\text{water velocity}_i) + g_3(\text{substrate size}_i) + g_4(\text{stream width}_i) + g_5(\text{bank angle}_i) + g_6(\text{bank vegetation cover}_i) + g_7(\text{distance to bank}_i) + g_8(\text{cell location}_i)$$

where $g(\cdot)$ is a nonparametric smooth function that characterizes the effect of an independent variable on the probability of response (Venables and Ripley 1994). The nonparametric functions for each of the independent variables were fitted using a loess smoothing function.

Spatial autocorrelation is a common statistical property of ecological variables and presents a problem for statistical testing because autocorrelated data violate the assumption of independence common to most standard statistical procedures (Legendre 1993; Thomson et al. 1996). We expected spatial autocorrelation within our study reaches because neighboring cells are likely to be more similar in habitat characteristics than are cells separated by a greater distance. One way to overcome this problem is to explicitly incorporate spatial structure into statistical models (Legendre 1993). Based on the approach used by Hobert et al. (1997), we incorporated spatial dependence by including a nonlinear spatial term ("cell location") on equal footing with the other independent variables. We defined cell location as the distance from the downstream-most transect of reach 1 (the downstream-most study reach) to the habitat transect containing the cell of interest. If spatial structure is important, the inclusion of the variable "cell location" would be expected to significantly increase the amount of variation explained by the model.

The best combination of independent variables was determined by evaluating the change in deviance (McCullagh and Nelder 1989) resulting from dropping each variable from the model in the presence of all other variables. Analysis of deviance and likelihood ratio tests (based on the binomial distribution) were used to test the significance of each of the independent variables on the probability of redd presence (McCullagh and Nelder 1989). The relative importance of significant variables was determined by calculating the Akaike information criteria (AIC; Linhart and Zucchini 1986). Larger AIC values indicate a greater relative importance. In order to evaluate the explanatory power provided by each independent variable, we also calculated the coefficient of determination (R^2) for each independent variable (Nagelkerke 1991). Values of R^2 were calculated by adding variables to the model in the order of their associated AIC value, such that the independent variable with the largest AIC value was added first and the variable with the smallest AIC value was added last.

Although nonparametric logistic regression is useful for evaluating the relative importance of independent variables and for describing the shape of response curves, parametric models are preferable in developing a predictive model if they provide an adequate fit to the data (Yee and Mitchell 1991). To develop a parametric model from the nonparametric model, we first examined plots of the response curves generated by the nonparametric regression for the four highly significant independent variables ($P < 0.0001$). Based on the shapes of these response curves, we determined the appropriate parametric model (e.g., linear, polynomial, logarithmic) to approximate the nonparametric functions. We based the parametric model solely on the highly significant independent variables to ensure that only those independent variables with high explanatory power were included in the model.

To assess whether the parametric model predicted the probability of use (p_i) as adequately as the nonparametric model (i.e., that

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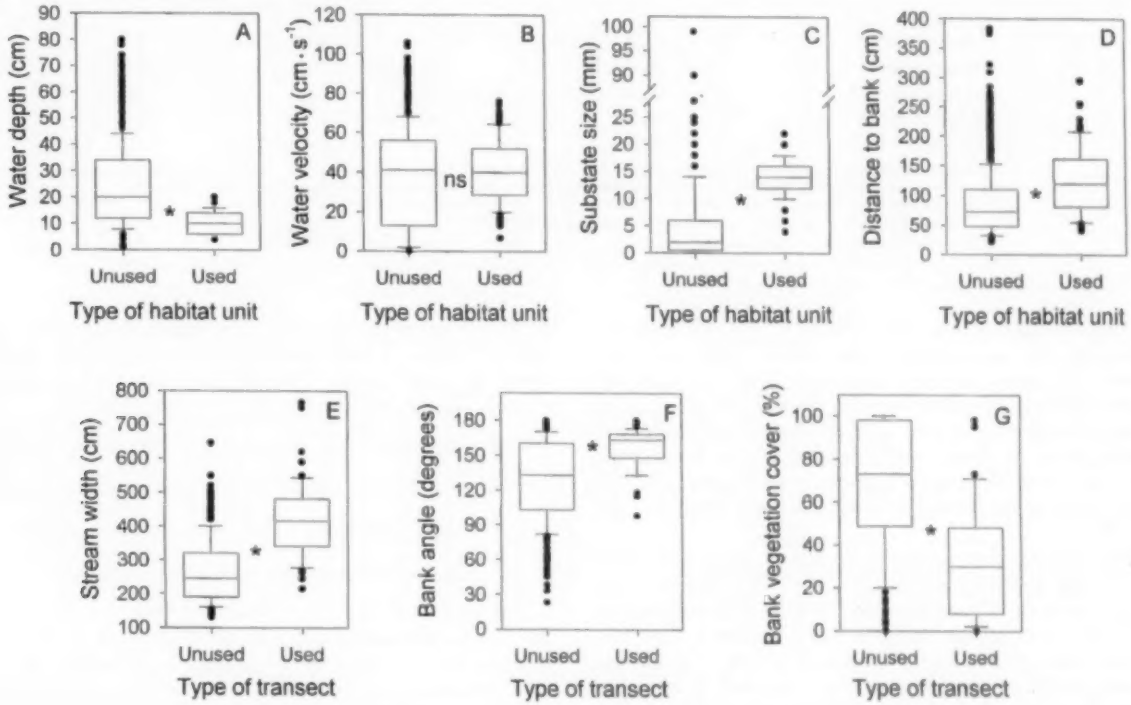
Fig. 2. Box plots showing (A) water depth, (B) water velocity, (C) substrate size, (D) distance to the nearest streambank, (E) stream width, (F) bank angle, and (G) bank vegetation cover for cells or transects that were unused versus used by spawning golden trout. The bottom and top of each box indicate the 25th and 75th percentiles, respectively, the line within each box marks the median, and the whiskers below and above each box indicate the 10th and 90th percentiles, respectively. Observations falling outside these percentiles are shown as points above and below the whiskers. An asterisk between boxes indicates that the difference between unused and used cells was highly significant ($P < 0.0001$), and "ns" indicates that the difference was not significant ($P > 0.05$). Significance was determined using a Kruskal–Wallis one-way analysis of variance. Data are from all 11 study reaches combined. For water depth, water velocity, substrate size, and distance to the nearest streambank, $n_{unused} = 1674$ and $n_{used} = 91$. For stream width, bank angle, and bank vegetation cover, $n_{unused} = 291$ and $n_{used} = 62$.



the functional forms that we used in the parametric model accurately represented the response curves generated by the nonparametric regression), the total deviances of the two models were compared. Since the fit of the two models was similar, we then used the parametric model to calculate, for each cell, the probability of use by spawning golden trout. All regression-related calculations were done using the S-Plus statistical package (MathSoft 1997).

Model cross-validation

To determine how well the parametric model fit data from stream reaches not used in model development, we used a cross-validation procedure (Breiman et al. 1984) followed by χ^2 goodness-of-fit tests (Hosmer and Lemeshow 1989). We first dropped one of the 11 study reaches from the data set and estimated model parameters using the remaining 10 reaches. We then used this reduced model to calculate the p_i values for all cells in the dropped reach. This procedure was repeated 10 times, each time dropping a different study reach from the model. We then divided all cells from each of the dropped reaches into 22 groups based on their predicted p_i values. The goodness-of-fit between the observed and predicted probabilities of redd presence for each these 22 groups was determined using the Pearson χ^2 statistic, which was then compared with a χ^2 distribution with 14 degrees of freedom (degrees of freedom = number of groups – number of parameters estimated in model = 22 – 8). A small P value indicates that the model does not provide a good fit to the data.

Visualization of model fit

To visualize the fit of the parametric model, we selected one of the 11 study reaches and compared the probability of use associated with each cell in this study reach with the locations actually used by spawning golden trout. For this analysis, we purposefully selected the study reach to which the model provided the poorest fit (reach 9), since this allowed a more thorough evaluation of the reasons underlying any discrepancies between p_i values and actual redd locations than if we had used a study reach to which the model provided a better fit.

Results

Golden trout spawning activity began on May 28 and only a few females were still observed on redds by June 5. No spawning was seen after June 8. Females constructed a total of 153 redds in the study reaches (0–67 per reach). Since each cell could contain more than one redd, the number of redd-containing cells in the study reaches was 91 (5%).

Average habitat values for cells used and not used by golden trout were significantly different for all measured

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Table 1. Analysis of deviance table showing the statistical significance (P value), AIC, and R^2 values of the independent variables in the nonparametric logistic regression model.

	Model		Test				
Model	Deviance	df	Deviance[a]	df[b]	P value	AIC[c]	R^2[d]
Null model	717	1764					
Full model	308	1747.6					62.0
Full model less:							
substrate size	457	1750.0	149	2.5	$<10^{-10}$	487.0	31.6
water depth	359	1748.6	51	1.0	$<10^{-10}$	391.8	49.4
water velocity	334	1749.2	26	1.6	9.5×10^{-7}	365.6	55.2
stream width	329	1749.5	21	1.9	2.5×10^{-5}	360.0	59.1
cell location	319	1751.3	11	3.7	0.02	346.4	60.3
vegetation cover	315	1749.4	7	1.8	0.02	346.2	61.2
bank angle	311	1749.2	3	1.6	0.11	342.6	61.6
distance to bank	310	1749.5	2	1.9	0.22	341.0	62.0

[a]Test deviance = (deviance of full model less one covariate) – (deviance of full model).
[b]Test df = (df of full model less one covariate) – (df of full model).
[c]AIC = (deviance of full model less one covariate) + 2(1765 – df of full model less one covariate).
[d]The R^2 value shown for each variable represents the R^2 of a model that includes the variable and all other variables with higher AIC scores.

variables except water velocity (Fig. 2). Cells used for spawning had on average significantly shallower water depths and larger substrates and were further from streambanks than unused cells (Figs. 2A, 2C, and 2D). In addition, transects containing at least one cell used for spawning were characterized by larger stream widths, larger bank angles (i.e., less bank overhang), and less bank vegetation cover than transects containing only unused cells (Figs. 2E, 2F, and 2G).

The overall nonparametric logistic regression model (eq. 2) was highly significant ($P < 10^{-10}$) and explained 62% of the variation in redd location (Table 1). The independent variables water depth, water velocity, substrate size, and stream width all had highly significant effects ($P < 0.0001$) on the probability of redd presence, while cell location and bank vegetation cover had marginally significant effects (Table 1). Bank angle and distance to bank did not contribute significantly to the model ($P > 0.05$) (Table 1). Based on the AIC (Table 1), the relative importance of the significant variables in explaining the location of redds was substrate size > water depth > water velocity > stream width > cell location > bank vegetation cover. The four highly significant independent variables alone accounted for 59% of the variation in redd location, while the two marginally significant independent variables, cell location and bank vegetation cover, together accounted for only an additional 2% of the variation (Table 1).

The relationships between p_i and the highly significant independent variables all had substantial nonlinear contributions (Fig. 3). The response shapes indicated that the probability of redd presence was highest in areas with intermediate substrate sizes (Fig. 3A), the shallowest water depths (Fig. 3B), intermediate water velocities (Fig. 3C), and the largest stream widths (Fig. 3D). We approximated these response shapes with second-degree polynomials (for substrate size, water depth, and water velocity) and a logarithmic function (for stream width). The difference in the deviance between the nonparametric and parametric regression models was not significant ($P > 0.05$), indicating that our choice of functional forms closely approximated the actual form of each significant relationship. The parametric model, based on water depth, velocity, substrate size, and stream width, is represented by the following equation:

$$\begin{aligned}(3)\quad \hat{\theta} = {} & -29.44 + 0.63\,(\text{water depth}) - 0.03\,(\text{water depth})^2 \\ & + 0.16\,(\text{water velocity}) - 0.002\,(\text{water velocity})^2 \\ & + 1.52\,(\text{substrate size}) - 0.05\,(\text{substrate size})^2 \\ & + 2.17\,(\log_e[\text{stream width}]).\end{aligned}$$

The results of the cross-validation procedure indicated that the parametric model generally provided a good fit to study reaches not used for model parameterization. For the 22 categories of the linear predictor, the observed and predicted probabilities of use were very similar, and almost all observed values were within the 95% confidence interval (Fig. 4). Despite the reasonably close match between observed and predicted probabilities, the χ^2 statistic indicated a poor fit of the model to the data (Pearson's $\chi^2 = 36.3$, df = 14, $P = 0.01$). Further examination of the data indicated that two of the 1765 cells (0.1%) had very large standardized residuals. Both cells were located in reach 9, and although these cells had very low predicted probabilities of containing redds, both were used by spawning fish. Both cells were associated with water depths, water velocities, and stream widths that were highly suitable for spawning but contained substrates that were finer than those typically used by spawning fish (substrate size = 4 and 6 mm) (Fig. 3). Although we had no a priori reason to exclude these cells from the data set (e.g., if the substrate sizes associated with these cells were the result of measurement error), it is nonetheless important to determine whether these two cells alone were responsible for the lack of model fit. When these two "outliers" were removed from the data set, the model fit was greatly improved (Pearson's $\chi^2 = 10.5$, $P = 0.72$).

A visualization of the model fit is provided by the stream channel map of reach 9 shown in Fig. 5. Female golden trout constructed redds in 15 of the 205 cells (7%). The poorer fit

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Fig. 3. Shape of the estimated nonparametric function (g) and 95% confidence intervals for each of the highly significant predictors of redd presence or absence: (A) substrate size, (B) water depth, (C) water velocity, and (D) stream width. These functional forms describe the effect of each independent variable on the probability of redd presence. Hatch marks above the x-axes indicate the measured values of each independent variable.

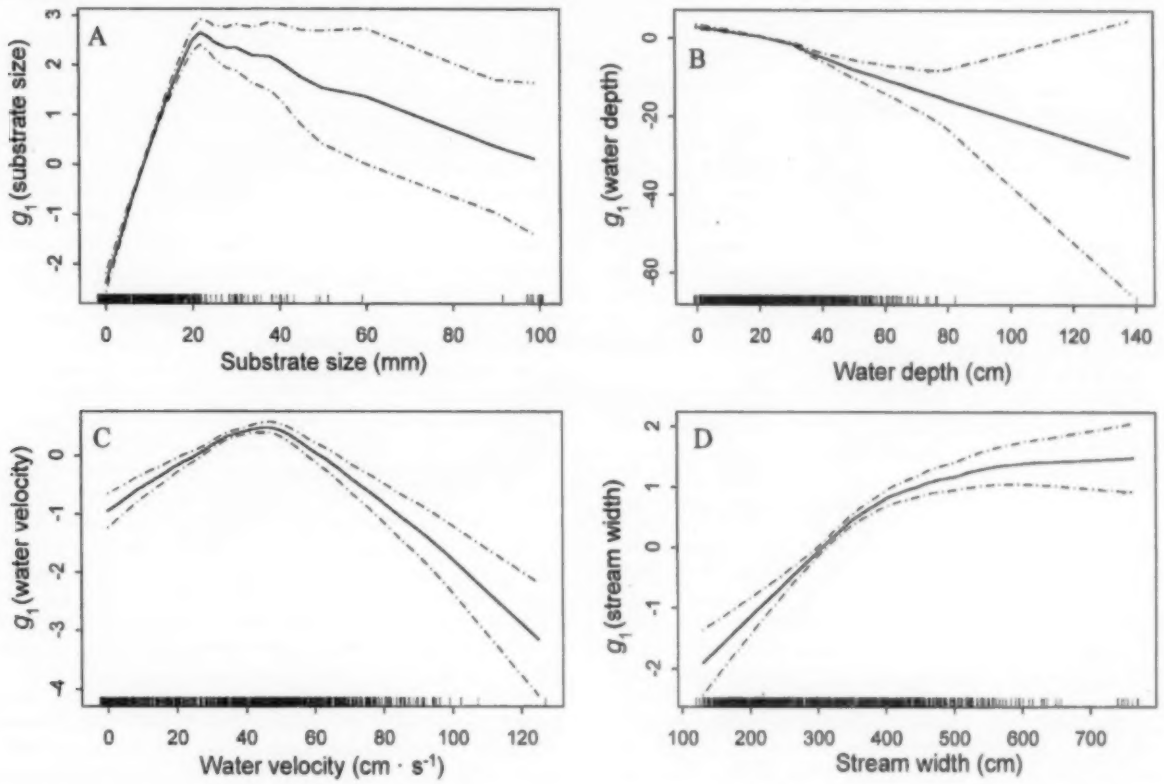
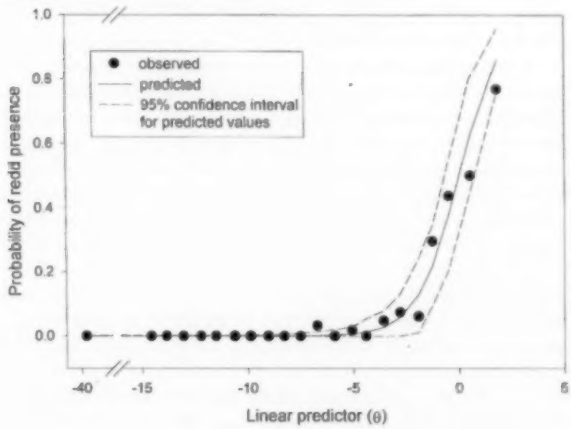


Fig. 4. Results of the cross-validation procedure showing the observed probability of redd presence (±95% confidence interval) and predicted probability of redd presence for 22 categories of the linear predictor (θ). The close association between the observed and predicted probabilities indicates that the model generally provided a good fit to the data from stream reaches not used in model parameterization.



of the model to reach 9 than to all other stream reaches was due to the two outlier cells that contained redds despite having very low predicted probabilities of redd presence ($\hat{p} < 0.2$) (Fig. 5). Despite these outliers, Fig. 5 indicates a close correspondence between the locations of cells with high predicted probabilities of use and the locations of cells that actually contained redds. For cells categorized as $\hat{p} \leq 0.25$, $0.25 < \hat{p} < 0.4$, or $\hat{p} \geq 0.4$, 1.1, 33.3, and 78.6%, respectively, actually contained redds.

Discussion

Our results indicate that locations chosen by spawning golden trout in Mulkey Creek were strongly influenced by water depth, water velocity, substrate size, and stream width. Of these four highly significant independent variables, substrate size, water depth, and water velocity alone explained the majority of the variation in redd location. This finding is in agreement with the results of other studies that also showed the importance of these three variables in discriminating between sites used and not used by spawning salmonids (Rinne 1980; Witzel and MacCrimmon 1983; Parsons and Hubert 1988; Grost et al. 1990; Thurow and King 1994; Knapp and Vredenburg 1996). The significant effect

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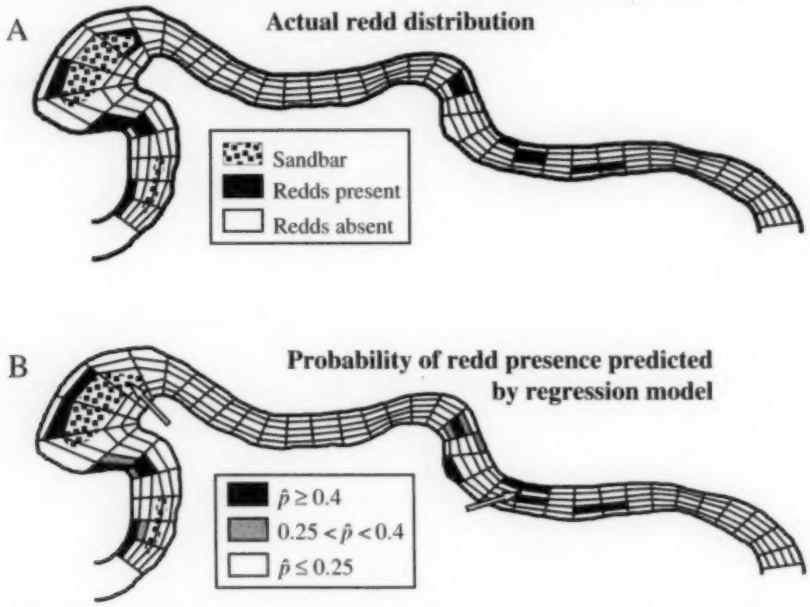
Fig. 5. Stream channel map of reach 9 showing (A) the locations of redd-containing cells and (B) the predicted probabilities of use by spawning golden trout for each cell, calculated using the parametric logistic regression equation. In Fig. 5B, the locations of two outliers described in the Results section are indicated by arrows. For scale, each cell is about 2.5 m long.



of water velocity in our model is perhaps surprising, since the average water velocity was not significantly different between those cells that were used by spawning golden trout and those that were not. However, the importance of water velocity to spawning trout is suggested by the fact that even though the average water velocity of used and unused cells was not significantly different, water velocities of used cells had a much narrower range than the water velocities of unused cells. This narrower range but similar mean water velocity indicates that spawning females avoided cells with the lowest and highest water velocities.

Stream width explained a relatively small, but highly significant, amount of the variation in redd location. Because the importance of stream width has not been previously reported, some discussion of the role of stream width in influencing spawning locations of golden trout in Mulkey Creek is warranted. Based on the same Mulkey Creek study area, the results of Knapp et al. (1998) indicated that all golden trout redds were constructed in wide stream reaches and they attributed this spatial distribution of redds to the fact that water depths, water velocities, and substrate sizes used by spawning golden trout were much more common in wide than in narrow reaches. While true, the significant effect of stream width in our model even after the effects of water depth, water velocity, and substrate size were accounted for suggests that female golden trout either used stream width (in addition to depth, velocity, and substrate) to select spawning locations or selected spawning locations based in part on additional habitat characteristics that were also associated with wide reaches but were not measured in our study.

Previous studies of spawning habitat preferences by stream salmonids have suggested additional factors beyond those measured in our study that may influence spawning locations (e.g., groundwater upwelling, river bottom topography; Witzel and MacCrimmon 1983; Shirvell 1989). It could be argued, therefore, that our study failed to provide a complete evaluation of all the important factors influencing choice of spawning location by golden trout. While incorporation of other variables, such as groundwater upwelling, might have increased the explanatory ability of our model, the fact that our models explained nearly 60% of the variation in redd location and accurately predicted the cell-specific probabilities of use indicates that our choice of habitat variables was sufficiently complete. In addition, we purposefully developed our habitat model based exclusively on easily measured variables to facilitate future model application. Because groundwater upwelling and river bottom topography are relatively difficult to quantify, we did not consider these variables in our study.

Our model provided a good fit to all but two of the 1765 cells used in this study, but the two exceptions are worthy of additional discussion. The cross-validation procedure identified two cells located in a single reach that contained redds despite having very low predicted probabilities of redd presence. This discrepancy is likely the result of within-cell variability in habitat characteristics. Although we attempted to minimize within-cell variability by using small cells (~0.75 m^2), observations made during measurement of habitat characteristics indicated that a small subset of cells still showed considerable within-cell variability. In these cases, habitat characteristics measured in the center of the cell may not have accurately reflected habitat conditions elsewhere in the cell. Because elimination of these two cells from the data set changed a previously poor fit to a very good fit, we conclude that while the parametric model did not adequately fit

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data from all 1765 cells, it did provide a very good fit to all 1674 cells that did not contain redds and to 89 of the 91 redd-containing cells (97.8%).

Spatial autocorrelation in data and colinearity between independent variables may be common in ecological studies (Legendre 1993; Thomson et al. 1996), including those investigating fish–habitat relationships (Mathur et al. 1985), and can pose substantial problems for regression analyses. In our study, spatial autocorrelation could result in an underestimation of the uncertainty associated with parameter estimates by reducing the independence of neighboring cells. We surmounted this problem by including the nonlinear spatial variable "cell location," following the methods of Legendre (1993) and Hobert et al. (1997). The fact that cell location was only marginally significant and increased the explained variation by only 1.6% suggests either that spatial dependence was relatively unimportant in understanding the factors influencing the choice of spawning location by female golden trout or that the habitat variables included in the model accounted for most of the spatial autocorrelation between neighboring cells. Colinearity between independent variables will cause the regression coefficient of any independent variable to depend on which other independent variables are included in the model (Neter et al. 1990). Because of the likely presence of correlations between independent variables in our model, the regression coefficients might change if the independent variables in the model were changed (e.g., by the addition of a new independent variable). Therefore, we interpreted the regression coefficients only in the presence of all other variables in the model.

Given the widely held belief that locations chosen by spawning stream salmonids are strongly influenced by water depth, water velocity, and substrate size (Bjornn and Reiser 1991), it is surprising that the single study that we were able to find that attempted to predict spawning locations based on these habitat characteristics indicated a very poor match between locations predicted as suitable for spawning and locations actually used by spawning fish (Shirvell 1989). This failure is in marked contrast with the ability of our model to accurately predict the cell-specific probability of use by spawning golden trout.

Our use of a GAM is a substantial improvement over previous approaches to modeling fish–habitat relationships. Past studies have relied on generalized linear models such as traditional logistic regression and have therefore needed to (*i*) assume a linear relationship between the logit of the dependent variable and each of the habitat variables (Thielke 1985), (*ii*) transform continuous variables into categorical variables (Thielke 1985), or (*iii*) incorporate nonlinear terms based on an iterative process that is blind to the actual shapes of the response curves (Bozek and Rahel 1991). Our use of a GAM allowed us to assess the significance of habitat variables without making assumptions about the shapes of the relationships between the independent variables and the probability of use. After using plots of the estimated nonparametric functions to evaluate the form of each significant relationship, we constructed a parametric logistic regression model that incorporated nonlinear terms chosen explicitly to approximate each relationship.

The results of our study indicate that the probabilities of golden trout in Mulkey Creek using a particular location for spawning are predictable based on easily measured microhabitat and channel characteristics. Since the habitats used by spawning golden trout in Mulkey Creek are qualitatively similar to habitats used by spawning golden trout in other streams (R.A. Knapp, personal observation) and to habitats used by several other salmonid species (Knapp and Vredenburg 1996), it seems likely that habitat use by spawning salmonids will generally be predictable using a model approach similar to the one that we used in this study. Further research will be necessary, however, to determine how generally applicable our particular model is to golden trout populations outside our study area and to other species of stream-dwelling salmonids.

In conclusion, although nonparametric regression approaches have only recently begun to be used in ecological studies (e.g., Wiser et al. 1998), our results suggest that they may have considerable utility in the development of models to predict fish distributions based on habitat characteristics. Given the escalating pace at which fish habitats are being altered, such models are increasingly important in predicting the effects of these alterations on populations.

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References

Beecher, H.A., Johnson, T.H., and Carleton, J.P. 1993. Predicting microdistributions of steelhead (*Oncorhynchus mykiss*) parr from depth and velocity preference criteria — test of an assumption of the Instream Flow Incremental Methodology. Can. J. Fish. Aquat. Sci. **50**: 2380–2387.

Bjornn, T.C., and Reiser, D.W. 1991. Habitat requirements of salmonids in streams. Am. Fish. Soc. Spec. Publ. **19**: 83–138.

Bovee, K.D. 1978. The incremental method of assessing habitat potential for coolwater species, with management implications. Am. Fish. Soc. Spec. Publ. **11**: 340–346.

Bozek, M.A., and Rahel, F.J. 1991. Assessing habitat requirements of young Colorado River cutthroat trout by use of macrohabitat and microhabitat analyses. Trans. Am. Fish. Soc. **120**: 571–581.

Breiman, L., Friedman, J.H., Olshen, R., and Stone, C.J. 1984. Classification and regression trees. Wadsworth, Belmont, Calif.

California Department of Fish and Game. 1996. Instream flow and habitat development investigations for Mill Creek, Mono County, California. Stream Evaluation Rep. 96-1, California Department of Fish and Game, Sacramento, Calif.

Cleveland, W.S., and Devlin, S.J. 1988. Locally weighted regression: an approach to regression analysis by local fitting. J. Am. Stat. Assoc. **83**: 596–610.

Efron, B., and Tibshirani, R. 1991. Statistical analysis in the computer age. Science (Washington, D.C.), **253**: 390–395.

© 1999 NRC Canada

Grost, R.T., Hubert, W.A., and Wesche, T.A. 1990. Redd site selection by brown trout in Douglas Creek, Wyoming. J. Freshwater Ecol. **5**: 365–371.

Hobert, J.P., Altman, N.S., and Schofield, C.L. 1997. Analyses of fish species richness with a spatial covariate. J. Am. Stat. Assoc. **92**: 846–854.

Hosmer, D.W., Jr., and Lemeshow, S. 1989. Applied logistic regression. John Wiley & Sons, New York.

Knapp, R.A., and Vredenburg, V.T. 1996. Spawning by California golden trout: characteristics of spawning fish, seasonal and daily timing, redd characteristics, and microhabitat preferences. Trans. Am. Fish. Soc. **125**: 519–531.

Knapp, R.A., Vredenburg, V.T., and Matthews, K.R. 1998. Effects of stream channel morphology on golden trout spawning habitat and recruitment. Ecol. Appl. **8**: 1104–1117.

Legendre, P. 1993. Spatial autocorrelation: trouble or a new paradigm? Ecology, **74**: 1659–1673.

Linhart, H., and Zucchini, W. 1986. Model selection. John Wiley & Sons, New York.

Lorenz, J.M., and Eiler, J.H. 1989. Spawning habitat and redd characteristics of sockeye salmon in the glacial Taku River, British Columbia and Alaska. Trans. Am. Fish. Soc. **118**: 495–502.

MathSoft. 1997. S-Plus user's manual, version 4. Data Analysis Products Division, MathSoft, Seattle, Wash.

Mathur, D., Bason, W.H., Purdy, E.J., Jr., and Silver, C.A. 1985. A critique of the Instream Flow Incremental Methodology. Can. J. Fish. Aquat. Sci. **42**: 825–831.

McCullagh, P., and Nelder, J.A. 1989. Generalized linear models. Chapman and Hall, London, U.K.

Nagelkerke, N.J.D. 1991. A note on a general definition of the coefficient of determination. Biometrika, **78**: 691–692.

Nehring, R.B., and Anderson, R.M. 1993. Determination of population-limiting critical salmonid habitats in Colorado streams using the Physical Habitat Simulation System. Rivers, **4**: 1–19.

Neter, J., Wasserman, W., and Kutner, M.H. 1990. Applied linear statistical models: regression, analysis of variance, and experimental designs. Irwin, Homewood, Ill.

Parsons, B.G.M., and Hubert, W.A. 1988. Influence of habitat availability on spawning site selection by kokanees in streams. N. Am. J. Fish. Manage. **8**: 426–431.

Platts, W.S., Megahan, W.F., and Minshall, G.W. 1983. Methods for evaluating stream, riparian, and biotic conditions. U.S. For. Serv. Gen. Tech. Rep. INT-138.

Reiser, D.W., Wesche, T.A., and Estes, C. 1989. Status of instream flow legislation and practices in North America. Fisheries (Bethesda), **14**: 22–29.

Rinne, J.N. 1980. Spawning habitat and behavior of Gila trout, a rare salmonid of the southwestern United States. Trans. Am. Fish. Soc. **109**: 83–91.

Rosgen, D.L. 1994. A classification of natural rivers. Catena, **22**: 169–199.

Shirvell, C.S. 1989. Ability of PHABSIM to predict chinook salmon spawning habitat. Regul. Rivers Res. Manage. **3**: 277–289.

Stalnaker, C.B. 1979. The use of habitat structure preferenda for establishing flow regimes necessary for maintenance of fish habitat. *In* The ecology of regulated streams. *Edited by* J.V. Ward and J.A. Stanford. Plenum Press, New York. pp. 321–337.

Stefferud, J.A. 1993. Spawning season and microhabitat use by California golden trout (*Oncorhynchus mykiss aguabonita*) in the southern Sierra Nevada. Calif. Fish Game, **79**: 133–144.

Thielke, J. 1985. A logistic regression approach for developing suitability-of-use functions for fish habitat. *In* Proceedings of the Symposium on Small Hydropower and Fisheries. *Edited by* F.W. Olson, R.G. White, and R.H. Hamre. American Fisheries Society, Aurora, Co. pp. 32–38.

Thomson, J.D., Weiblen, G., Thomson, B.A., Alfaro, S., and Legendre, P. 1996. Untangling multiple factors in spatial distributions: lilies, gophers, and rocks. Ecology, **77**: 1698–1715.

Thurow, R.F., and King, J.G. 1994. Attributes of Yellowstone cutthroat trout redds in a tributary of the Snake River, Idaho. Trans. Am. Fish. Soc. **123**: 37–50.

Venables, W.N., and Ripley, B.D. 1994. Modern applied statistics with S-Plus. Springer-Verlag, New York.

Wiser, S.K., Peet, R.K., and White, P.S. 1998. Prediction of rare-plant occurrence: a Southern Appalachian example. Ecol. Appl. **8**: 909–920.

Witzel, L.D., and MacCrimmon, H.R. 1983. Redd-site selection by brook trout and brown trout in southwestern Ontario streams. Trans. Am. Fish. Soc. **112**: 760–771.

Yee, T.W., and Mitchell, N.D. 1991. Generalized additive models in plant ecology. J. Veg. Sci. **2**: 587–602.

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Effect of maternal growth history on egg number and size in wild white-spotted char (*Salvelinus leucomaenis*)

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Abstract: Differences in egg number and egg size within a wild population of white-spotted char (*Salvelinus leucomaenis*) were examined from the perspective of maternal growth history by using a back-calculation method. Relatively greater egg size variation occurred among females (61.6%) than within females (38.4%). Larger females produced a larger number of eggs, which were also larger in size. To understand the causes of variation in egg number and egg size, several contributory factors determining maternal size were considered (initial size, growth rate for each age, total age). Egg number was not associated with maternal growth history. Contrary to egg number, egg size was strongly associated with maternal growth history; high growth in the first year was negatively associated with egg size, but high growth in the second year was positively associated with egg size. The results indicated that white-spotted char potentially exhibit phenotypic plasticity in egg size according to their growth history.

Résumé : Les différences dans la quantité et la grosseur des œufs pour une population sauvage d'omble à taches blanches (*Salvelinus leucomænis*) ont été observées du point de vue des antécédents de croissance maternels par une méthode de rétrocalcul. La fluctuation dans la taille des œufs était relativement plus élevée parmi les femelles (61,6 %) que chez la même femelle (38,4 %). Les plus grosses femelles pondaient des œufs plus gros et en plus grand nombre. Pour comprendre les causes de ces variations dans le nombre et la grosseur des œufs, plusieurs facteurs contribuant à déterminer la taille de la mère ont été étudiés (taille initiale, taux de croissance pour chaque âge, âge total). Le nombre d'œufs n'était pas associé aux antécédents de croissance maternels; cependant, la taille des œufs leur était fortement associée. Une forte croissance maternelle pendant la première année était négativement associée à la taille des œufs, tandis que l'association était positive dans le cas d'une forte croissance maternelle pendant la deuxième année. Les résultats montrent que l'omble à taches blanches présente une plasticité phénotypique dans la grosseur de ses œufs qui est fonction de ses antécédents de croissance.

[Traduit par la Rédaction]

Introduction

Many salmonid species possess considerable variation in maternal reproductive characters, such as egg number and egg size, both among (Fleming and Gross 1990; Quinn et al. 1995) and within populations (Thorpe et al. 1984; van den Berghe and Gross 1989; Jonsson et al. 1996). While egg number might well be expected to increase with maternal size, simply because of geometric constraints, there has also been a considerable accumulation of evidence that egg size also increases with maternal size (e.g., Jonsson and Hinder 1982; Thorpe et al. 1984; Beacham and Murray 1985; van den Berghe and Gross 1989). The relationships between egg size and maternal size in general, however, were much weaker than the relationships between egg number and maternal size.

An experimental study by Bagenal (1969) showed that low-ration brown trout (*Salmo trutta*) produced smaller numbers of eggs with larger size than high-ration brown trout after controlling for gonad weight. Low-ration rainbow trout (*Oncorhynchus mykiss*) have also been found to produce fewer eggs after controlling for female size (Scott 1962). However, egg number in rainbow trout denied food for 4 months prior to spawning was similar to that in normally fed rainbow trout after controlling for female size (Kato 1975). In addition, Kato (1975) suggested that egg size increased with age rather than with maternal size because a correlation of egg size with body size was unlikely within the same age group. In Atlantic salmon (*Salmo salar*) and sockeye salmon (*Oncorhynchus nerka*), smolt age and (or) sea age were also considered as a predictor of egg production (Thorpe et al. 1984; Kaeriyama et al. 1995). Thorpe

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et al. (1984) found that Atlantic salmon that developed rapidly in freshwater (i.e., they had a younger age at smolting) produced larger numbers of eggs with smaller size after controlling for female size.

Intrapopulational variations in life history traits, such as age at maturity, egg size, migration time and breeding season, have often been considered to result from adaptive phenoypic plasticity (Hutchings 1996; Kitano 1996; Quinn and Adams 1996). Large female coho salmon (*Oncorhynchus kisutch*) are able to obtain better spawning sites through competition and dig deeper nests, hence providing greater parental care than small females (van den Berghe and Gross 1984, 1989). By this mechanism, the optimal egg size in salmonids can be expected to increase with maternal size (Sargent et al. 1987). However, the result obtained by Holtby and Healey (1986) did not support maternal size dependence of egg survival. In Atlantic salmon, Jonsson et al. (1996) found that high growth rate during the freshwater period induced smaller eggs. They suggested that Atlantic salmon exhibited phenotypic plasticity in response to feeding opportunities experienced by the female before marine entry (i.e., females assessed the likely feeding opportunities to be encountered by their offspring).

As mentioned above, there have been various studies reporting correlations between both egg size and egg number and three factors (body size, age, growth rate). However, there have been few efforts to devise comprehensive models of these phenomena. Body size results from the combination of growth rates during successive developmental stages and age. It is known that the growth rate during any given developmental stage is dependent on the growth rates during previous stages (Jobling and Koskela 1996; Nicieza and Metcalfe 1997). To clarify the causes of variation in egg number and size, it is necessary to understand the relationships among body size, age, and growth rate of individual fish. The objective of this study was to relate causes of intrapopulational variations in egg number and size of landlocked white-spotted char (*Salvelinus leucomaenis*) to maternal growth history.

Materials and methods

White-spotted char are distributed throughout Hokkaido Island, Japan (Ishigaki 1984). The present investigation was conducted in the Haraki River on the southern part of Hokkaido Island (41°44′N, 141°02′E) in 1996. The river is characterized by several impassable erosion-control dams, above which most fish have a nonmigratory life cycle. Females are iteroparous and most (88%) reach sexual maturity at age 3 years, the maximum age of females in this population being 5 years (K. Morita and S. Yamamoto, unpublished data). We established the study area above an erosion-control dam. We collected white-spotted char randomly using a backpack electrofisher (Smith-Root, Inc.) in the middle of October, the main spawning period of the species (Ishigaki 1984). We determined sex and maturity stage of each sample according to the condition of the ovary and the visceral cavity (Takashima et al. 1995). We also measured fork length (millimetres) and established age from otolith analysis (Yamamoto et al. 1992; Lai et al. 1996). Of the 121 fish captured, 22 were mature "ripe" females. Mature females ranged in age from 3 to 5 years.

Estimation of growth patterns of mature females

We estimated the former growth pattern of each mature female by a back-calculation method based on otolith radius and annulus radius measurements (Lai et al. 1996). The otolith annulus of white-spotted char begins to form from late May through June each year (Yamamoto et al. 1992).

There was a strong linear relationship between the natural logarithms of fork length and otolith radius ($r^2 = 0.98$, $P < 0.001$, $n = 121$). The relationship was described by

$$(1) \qquad \ln \mathrm{FL} = (1.251 \ln R) - 4.069$$

where FL is fork length (millimetres) at the time of capture and R is the total otolith radius. The logarithm of fork length was proportional to the logarithm of otolith radius, and as a result, we used a modified Fraser–Lee formula for the back-calculation of past fork length at time t (Bartlett et al. 1984):

$$(2) \qquad \ln \mathrm{FL}_t = (\ln R_t / \ln R)(\ln \mathrm{FL} - a) + a$$

where FL_t is fork length at time t, R_t is the annulus t radius length, and a is the intercept of the regression of ln R on ln FL. The intercept for the calculation is given in eq. 1, $a = 4.069$.The specific growth rate (G) was defined by the following equation (Ricker 1979):

$$(3) \qquad G = \frac{\ln \mathrm{FL}_2 - \ln \mathrm{FL}_1}{t_2 - t_1}$$

where FL_1 and FL_2 are fork lengths at times t_1 and t_2, respectively. We calculated three specific growth rates per year for each mature female. Consequently, the fork length of each mature female at age T (FL_T) could be considered on the basis of contributory factors as follows:

$$(4) \qquad \ln \mathrm{FL}_T = \ln \mathrm{FL}_0 + G_1 + G_2 + G_{3+}(T - 2)$$

where FL_0 is fork length at age 0 (fork length at first ring; $\mathrm{FL}_0 \equiv$ emergence size), G_1, G_2, and G_{3+} are specific growth rates from age 0 to age 1, from age 1 to age 2, and from age 2 to the time of capture, respectively, and T denotes the total age of the mature female ($T = 3$, 4, or 5 years). In this way, we considered that initial size (FL_0), growth rates (G_1, G_2, and G_{3+}) and age (T) make up the fork length (FL_T): $\mathrm{FL}_T = f(\mathrm{FL}_0, G_1, G_2, G_{3+}, T)$.

Measurements of egg number and egg size

We obtained the eggs by dissection and the egg number for each mature female was counted. Projective areas of all eggs were measured after water hardening using NIH image software (W. Rasband, National Institutes of Health, Bethesda, Md.). Egg size (mm) was calculated from the projective area to diameter by the following formula:

$$(5) \qquad \text{Egg size} = \sqrt{\frac{\text{Egg area (mm}^2\text{)}}{\pi}} \times 2.$$

Statistical analyses

We used model II analysis of variance to quantify the magnitudes of variation in egg size among females. Simple regression analysis was used to examine the relationships between fork length and either egg size or egg number. The relational predictors for variation in egg size and egg number were selected from the components of maternal size (FL_0, G_1, G_2, G_{3+}, T) by stepwise multiple regression using the forward selection procedure. Path analysis was also used to examine the relationship between predictor variables and either egg size or egg number. Natural logarithmic transformations were used to normalize data prior to analyses, except for the specific growth rates.

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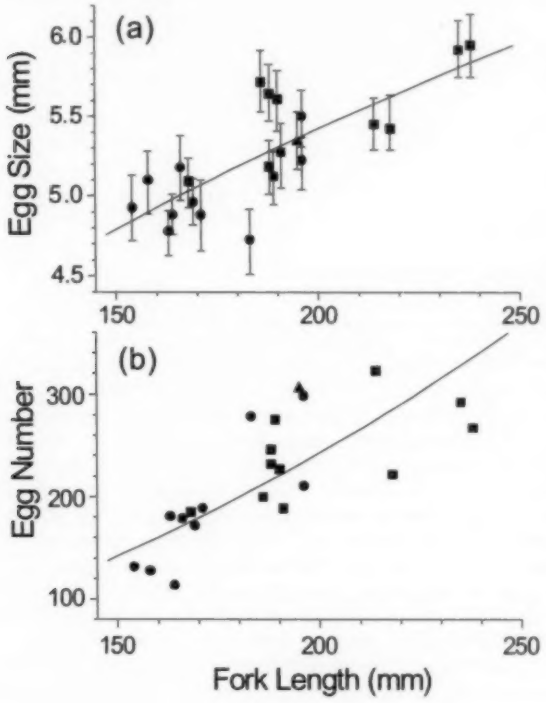
Fig. 1. Relationship between fork length and mean (*a*) egg size and (*b*) egg number. The lengths of error bars indicate interquartile ranges, i.e., the ends of the bars represent 25 and 75%. Symbols indicate age 3 (circles), age 4 (squares), and age 5 (triangles). Figure 1*a* regression line: ln(egg size) = 0.428 ln(fork length) – 0.575, $r^2 = 0.60$, $n = 22$, $P < 0.001$. Figure 1*b* regression line: ln(egg number) = 1.87 ln(fork length) – 4.39, $r^2 = 0.60$, $n = 22$, $P < 0.001$.



Results

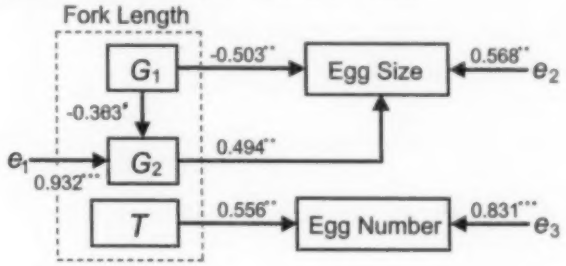
Egg sizes within individuals were distributed unimodally. The proportion of egg size variation among females was 61.6% and within females was 38.4% (one-way model II ANOVA: $F = 316.8$, df = 21,4319, $P < 0.001$). Relatively greater egg size variation occurred among females than within females.

Both mean egg size and egg number were positively correlated with fork length (Fig. 1). About 60% of both egg size and egg number variation among females was explained by variation in fork length (FL_T). Since the explanatory variable fork length (FL_T) was determined by initial size (FL_0), specific growth rates (G_1, G_2, G_3), and total age (T) (see eq. 4), stepwise multiple regression was conducted in order to seek the main predictors determining egg size and egg number. Stepwise multiple regression identified both G_1 and G_2 as the best predictors of egg size variation and only T as the best predictor of egg number variation (Table 1).

Path model construction

Path analysis is especially well suited for the study when the predictor variables have a presumed cause and effect relationship (Li 1986; Sokal and Rohlf 1995). To examine the potential causes of variation in egg size and egg number, we illustrated a path diagram using the main predictors identified by the stepwise multiple regression (Fig. 2). The criterion variable for egg size was affected by both G_1 and G_2. It is well known that the growth rate during any given developmental period is affected by the growth rates during previous periods (Jobling and Koskela 1996; Nicieza and Metcalfe 1997). Hence, G_1 could influence G_2, not vice versa. On the other hand, the criterion variable for egg number was affected by only T.

Fig. 2. Path diagrams illustrating the relationships among egg size, egg number, and components of fork length picked up by the stepwise multiple regression. Simple inductive causal relationships are indicated by arrows, G_1 and G_2 denote specific growth rates from age 0 to age 1 and from age 1 to age 2, respectively, T denotes total age, numerical values on the arrows indicate path coefficients, and e_1, e_2, and e_3 are residual variables. $^{***}P < 0.001$; $^{**}P < 0.01$; $^{*}P < 0.10$.



Path analysis showed that about 68% of egg size variation could be explained by variations in both G_1 and G_2. Moreover, the effect of G_1 was greatest among the components of fork length. Altogether, G_1 had a 44% negative effect and G_2 had a 24% positive effect on egg size. The effect of G_1 on G_2 was negative and marginally significant. With respect to egg number, the simple path diagram showed that about 31% of the variation could be explained by variations in T.

Discussion

A positive relationship between maternal size and offspring has been reported for a wide range of organisms (reviewed by Roff 1992). This pattern is particularly pronounced among salmonids (e.g., Jonsson and Hindar 1982; Thorpe et al. 1984; Beacham and Murray 1985; van den Berghe and Gross 1989; Kaeriyama et al. 1995; Jonsson et al. 1996).

Individuals of similar body size can have varying growth histories, including yearly growth rate, age at smolting, length of sea-run period, and total age. Maternal growth has often been used to explain egg size variation (Thorpe et al. 1984; Jonsson et al. 1996).

In this study, the previous growth history was considered based on yearly specific growth rates and total age and synthetic causal paths analyzed to explain egg size and egg number variation. Egg number was defined on the basis of maternal age (T), being a convenient criterion of body size, and egg size on the basis of the specific growth rates (G_1 and G_2). Contributory factors for egg size, however, cannot be obvious criteria of maternal size due to the negative ef-

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Table 1. Summary of stepwise multiple regressions: egg size and egg number versus components of fork length (FL_0, G_1, G_2, G_{3+}, T).

Dependent variable		Partial correlation coefficient	Regression coefficient	P
Egg size	Predictor variables			
	Intercept		1.711	<0.001
	G_1	–0.636	–0.196	<0.001
	G_2	0.629	0.324	0.002
	Remaining variables			
	FL_0	0.267		0.254
	G_{3+}	–0.278		0.236
	T	0.356		0.123
Egg number	Predictor variables			
	Intercept		4.696	<0.001
	T	0.556	0.714	0.007
	Remaining variables			
	FL_0	0.371		0.098
	G_1	–0.165		0.475
	G_2	0.239		0.297
	G_{3+}	–0.135		0.559

fect of G_1 and because the effects of each contributory factor (FL_0, G_1, G_2, G_{3+}, T) on maternal size were apparently positive (see eq. 4). Therefore, it can be concluded that egg number is determined by the maternal size but it cannot simply be claimed that the maternal size explains egg size.

The unexpected finding in this study is that the most important cause of egg size variation was early growth rate (G_1), the effect of such being negative. Theoretical (Sibly and Calow 1985) and empirical studies (Hutchings 1991) deduced the adaptive significance of an inverse relationship between offspring size and early feeding opportunity. If it is assumed that a correlation exists between growth condition during the early life of both the maternal parent and offspring, the production of larger eggs demonstrates adaptive plasticity, the mother having experienced low initial growth due to a less than optimal environment. Such an inference has been previously documented and demonstrated (e.g., Boersma 1995; Jonsson et al. 1996; Lobón-Cerviá et al. 1997).

The genetic control of variation in egg size and growth rate has been also documented in many studies (e.g., Kinghorn 1983; Nilsson 1994; Su et al. 1996, 1997). However, the heritability of body size during the first year is low (approximate range from 0.1 to 0.2) (Kinghorn 1983; Nilsson 1994; Su et al. 1996), whereas many studies have explained the variation in juvenile size by environmental heterogeneity, including such factors as temperature and population density (e.g., Elliott 1993; Bohlin et al. 1994). Hence, we might conclude that the growth rate in the first year was primarily determined by environmental rather than genetic factors. On the other hand, heritability of body size generally tends to be higher at older ages (reviewed by Kinghorn 1983), and a positive genetic correlation between egg size and maternal size was reported for rainbow trout (Su et al. 1997). In contrast with the G_1 effect, the G_2 effect may have a genetic component.

A relationship between maternal growth history and egg size was recently disputed by Jonsson et al. (1996) for an anadromous salmonid species, Atlantic salmon. In that study, due to a complicated anadromous life history, individual fish experienced diverse ecological episodes prior to spawning. In such a case, it would be difficult to construct a model explaining egg size variation based on maternal growth history information. In the present study, the problem of egg size variation influencing maternal growth history was applied by the use of landlocked white-spotted char with a simple life history. The successive annual growth rates and age provided both necessary and sufficient information for describing maternal size. Thus, the well-known superficial correlation between egg size and maternal size was able to be formally translated into the path analysis.

To our knowledge, most previous studies have implicitly assumed that body size variation was the primary cause of egg size variation, the effect being positive. However, both negative correlation and no correlation between egg size and maternal size occur in some salmoniformes (e.g., Iguchi and Yamaguchi 1994; Katayama et al. 1996). The analysis conducted in this study may be a useful tool in explaining such a phenomenon in addition to precise experimental manipulation.

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References

Bagenal, T.B. 1969. The relationship between food supply and fecundity in brown trout *Salmo trutta* L. J. Fish Biol. **1**: 167–182.

Bartlett, J.R., Randerson, P.F., Williams, R., and Ellis, D.M. 1984. The use of analysis of covariance in the back-calculation of growth in fish. J. Fish Biol. **24**: 201–213.

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Beacham, T.D., and Murray, C.B. 1985. Effect of female size, egg size, and temperature on developmental biology of chum salmon (*Oncorhynchus keta*) from the Nitinat River, British Columbia. Can. J. Fish. Aquat. Sci. **42**: 1755–1765.

Boersma, M. 1995. The allocation of resources to reproduction in *Daphnia galeata*: against the odds? Ecology, **76**: 1251–1261.

Bohlin, T., Dellefors, C., Faremo, U., and Johlander, A. 1994. The energetic equivalence hypothesis and the relation between population density and body size in stream-living salmonids. Am. Nat. **143**: 478–493.

Elliott, J.M. 1993. A 25-year study of production of juvenile seatrout, *Salmo trutta*, in an English Lake District stream. Can. Spec. Publ. Fish. Aquat. Sci. No. 118. pp. 109–122.

Fleming, I.A., and Gross, M.R. 1990. Latitudinal clines: a trade-off between egg number and size in Pacific salmon. Ecology, **71**: 1–11.

Holtby, L.B., and Healey, M.C. 1986. Selection for adult size in female coho salmon (*Oncorhynchus kisutch*). Can. J. Fish. Aquat. Sci. **43**: 1946–1959.

Hutchings, J.A. 1991. Fitness consequences of variation in egg size and food abundance in brook trout *Salvelinus fontinalis*. Evolution, **45**: 1162–1168.

Hutchings, J.A. 1996. Adaptive phenotypic plasticity in brook trout, *Salvelinus fontinalis*, life histories. Ecoscience, **3**: 25–32.

Iguchi, K., and Yamaguchi, M. 1994. Adaptive significance of inter- and intrapopulational egg size variation in ayu *Plecoglossus altivelis* (Osmeridae). Copeia, **1994**: 184–190.

Ishigaki, K. 1984. Exploring the mystery of charrs. Iwanami-shoten, Tokyo. (In Japanese.)

Jobling, M., and Koskela, J. 1996. Interindividual variations in feeding and growth in rainbow trout during restricted feeding and in a subsequent period of compensatory growth. J. Fish Biol. **49**: 658–667.

Jonsson, B., and Hinder, K. 1982. Reproductive strategy of dwarf and normal Arctic charr (*Salvelinus alpinus*) from Vangsvatnet Lake, western Norway. Can. J. Fish. Aquat. Sci. **39**: 1404–1413.

Jonsson, N., Jonsson, B., and Fleming, I.A. 1996. Does early growth cause a phenotypically plastic response in egg production of Atlantic salmon? Funct. Ecol. **10**: 89–96.

Kaeriyama, M., Urawa, S., and Fukuwaka, M. 1995. Variation in body size, fecundity, and egg size of sockeye salmon and kokanee salmon, *Oncorhynchus nerka*, released from hatchery. Sci. Rep. Hokkaido Salmon Hatchery, **49**: 1–9.

Katayama, S., Hishida, M., and Okata, A. 1996. Morphometric and reproductive characteristics of the pond smelt population in Lake Ogawara. Tohoku J. Agric. Res. **46**: 125–140.

Kato, T. 1975. The relation between the growth and reproductive characters of rainbow trout, *Salmo gairdneri*. Bull. Freshwater Fish. Res. Lab. (Tokyo), **25**: 83–115. (In Japanese with English summary.)

Kinghorn, B.P. 1983. A review of quantitative genetics in fish breeding. Aquaculture, **31**: 283–304.

Kitano, S. 1996. Size-related factors causing individual variation in seasonal reproductive success of fluvial male Dolly Varden (*Salvelinus malma*). Ecol. Freshwater Fish, **5**: 59–67.

Lai, H.L., Gallucci, V.F., and Gunderson, D.R. 1996. Age determination in fisheries: methods and application to stock assessment. *In* Stock assesment. *Edited by* V.F. Gallucci, S.B. Saila, D.J. Gustafson, and B.J. Rothschild. CRC Press, New York. pp. 82–178.

Li, C.C. 1986. Path analysis — a primer. 4th ed. The Boxwood Press, Pacific Grove, Calif.

Lobón-Cerviá, J., Utrilla, C.G., Rincón, P.A., and Amezcua, F. 1997. Environmentally induced spatio-temporal variations in the fecundity of brown trout *Salmo trutta* L.: trade-offs between egg size and number. Freshwater Biol. **38**: 277–288.

Nicieza, A.G., and Metcalfe, N.B. 1997. Growth compensation in juvenile Atlantic salmon: responses to depressed temperature and food availability. Ecology, **78**: 2385–2400.

Nilsson, J. 1994. Genetics of growth of juvenile Arctic char. Trans. Am. Fish. Soc. **123**: 430–434.

Quinn, T.P., and Adams, D.J. 1996. Environmental change affecting the migratory timing of American shad and sockeye salmon. Ecology, **77**: 1151–1162.

Quinn, T.P., Hendry, A.P., and Wetzel, L.A. 1995. The influence of life history trade offs and the size of incubation gravels on egg size variation in sockeye salmon (*Oncorhynchus nerka*). Oikos, **74**: 425–438.

Ricker, W.E. 1979. Growth rates and models. *In* Fish physiology. Vol. 8. *Edited by* W.S. Hoar, D.J. Randall, and J.R. Brett. Academic Press, New York. pp. 677–743.

Roff, D.A. 1992. The evolution of life histories. Chapman and Hall, New York.

Sargent, R.C., Taylor, P.D., and Gross, M.R. 1987. Parental care and the evolution of egg size in fishes. Am. Nat. **129**: 32–46.

Scott, D.P. 1962. Effect of food quantity on fecundity of rainbow trout, *Salmo gairdneri*. J. Fish. Res. Board Can. **19**: 715–731.

Sibly, R., and Calow, P. 1985. Classification of habitats by selection pressures: a synthesis of life-cycle and *r*/*K* theory. *In* Behavioural ecology. *Edited by* R.M. Sibly and R.H. Smith. Blackwell, Oxford, U.K. pp. 75–90.

Sokal, R.R., and Rohlf, F.J. 1995. Biometry. 3rd ed. W.H. Freeman and Company, New York.

Su, G.-S., Liljedahl, L.-E., and Gall, G.A.E. 1996. Genetic and environmental variation of body weight in rainbow trout (*Oncorhynchus mykiss*). Aquaculture, **143**: 71–80.

Su, G.-S., Liljedahl, L.-E., and Gall, G.A.E. 1997. Genetic and environmental variation of female reproductive traits in rainbow trout (*Oncorhynchus mykiss*). Aquaculture, **154**: 115–124.

Takashima, Y., Takada, T., Kudo, K., Matsumura, H., and Minowa, N. 1995. Age-specific maturation ratio of white-spotted charr *Salvelinus leucomaenis* in Furuu River in Hokkaido. Proc. Hokkaido Tokai Univ. **8**: 51–60. (In Japanese with English summary.)

Thorpe, J.E., Miles, M.S., and Keay, D.S. 1984. Developmental rate, fecundity and egg size in Atlantic salmon, *Salmo salar* L. Aquaculture, **43**: 289–305.

van den Berghe, E.P., and Gross, M.R. 1984. Female size and nest depth in coho salmon (*Oncorhynchus kisutch*). Can. J. Fish. Aquat. Sci. **41**: 204–206.

van den Berghe, E.P., and Gross, M.R. 1989. Natural selection resulting from female breeding competition in Pacific salmon (coho: *Oncorhynchus kisutch*). Evolution, **43**: 125–140.

Yamamoto, S., Nakano, S., and Tokuda, Y. 1992. Variation and divergence of the life history of Japanese charr *Salvelinus leucomaenis* in an artificial lake – inlet stream system. Jpn. J. Ecol. **42**: 149–157. (In Japanese with English summary.)

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A novel method for the estimation of age and growth in rajids using caudal thorns

M. Gallagher and C.P. Nolan

Abstract: The caudal thorns of four bathyrajid species from the Falkland Islands exhibit well-defined surface ridges. The apparent periodicity of ridge deposition suggests that caudal thorns exhibiting this sculpture have potential for use as ageing devices. Caudal thorns and vertebrae were removed from selected specimens. Thorn surface band counts were compared with counts from longitudinal sections of vertebral centra. Structures were read three times by each of three readers in a double-blind study. Differences in precision were found between readers. Estimates made by the most experienced reader showed least variation. A consistent difference between thorn and centrum band counts was recorded in the males of two species, with centrum counts lower than thorn counts by one growth increment. These differences were attributed to difficulties in interpreting the centrum sections of these species. In all other groups, counts were highly correlated with no significant difference between group means. Caudal thorns represent a novel ageing structure that provides estimates with precision similar to that of more conventional vertebral ageing techniques. Centrum and thorn edge analysis, supported by evidence from a tetracycline-marked individual of a related species, confirms the nature of band formation in both structures. The use of thorns as ageing structures, where appropriate, has the potential to be of significant benefit to the resource assessment and management of skate and ray fisheries.

Résumé : Les épines caudales de quatre espèces de Rajidae des Malouines ont à leur surface des crêtes bien définies. La périodicité apparente du dépôt de ces crêtes laisse penser que les épines caudales portant ces structures pourraient servir à la détermination de l'âge des poissons. On a prélevé les épines caudales et les vertèbres de spécimens choisis. On a comparé les comptes des bandes présentes à la surface des épines aux comptes des bandes de coupes longitudinales des corps vertébraux. Dans le cadre d'une étude à double insu, les structures ont été comptées trois fois par chacune des trois personnes impliquées. On a observé des différences dans la précision des mesures prises par ces personnes. Les estimations réalisées par la personne la plus expérimentée ont montré la plus faible variation. On a obtenu régulièrement une différence entre les comptes des bandes des épines et des corps vertébraux chez les mâles de deux espèces, les comptes des corps vertébraux étant inférieurs d'un incrément de croissance à ceux des épines. Ces différences étaient attribuables aux difficultés inhérentes à l'interprétation des coupes des corps vertébraux de ces espèces. Dans tous les autres groupes, les comptes étaient fortement corrélés et il n'y avait pas de différence significative entre les moyennes des groupes. Les épines caudales sont des structures employées depuis peu pour la détermination de l'âge qui fournissent des estimations aussi précises que les techniques plus classiques utilisant les vertèbres. Les résultats des analyses des corps vertébraux et du bord des épines, combinés à ceux obtenus chez un individu d'une espèce apparentée marqué à la tétracycline, confirment la nature de la formation des bandes sur ces deux structures. L'utilisation des épines comme structures pour la détermination de l'âge peut, quand elle est indiquée, présenter de grands avantages dans l'évaluation des stocks de raies et la gestion de leur pêche.

[Traduit par la Rédaction]

Introduction

The determination of age in commercially important fishes is an important component of fisheries resource assessment (Younger 1975; Casselman 1983). Growth rates, longevity, cohort structure, and ages at other significant periods within the life cycle (maturation, migration, etc.) can be determined and subsequently used in stock assessment and population models.

Due to the commercial importance of rajiformes, age and growth studies have been investigated in this group since the early part of this century (e.g., Steven 1936; Ishiyama 1951). Ageing rajiformes as with most elasmobranch species has historically been accomplished through the analysis of band formation in the vertebral centra (Ishiyama 1951, 1958; Daiber 1960; Taylor and Holden 1964; Du Buit 1972; Cailliet et al. 1983*a*; Waring 1984; Abdel-Aziz 1992; Zeiner and Wolf 1993). To date, few studies have validated the temporal nature of bands observed in vertebral centra (Holden and Vince 1973; Ryland and Ajayi 1984; Natanson 1993), although in many instances, validation often proves unfeasible due to the practical difficulties of recapturing marked animals and the facilities needed for laboratory growth studies.

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Verification is often the only method of assessing the temporal periodicity of band formation. Derived ages have been verified using marginal increment analysis of centra (e.g., Ishiyama 1951; Waring 1984; Abdel-Aziz 1992) and length–frequency analysis (e.g., Brander and Palmer 1985; Smith and Merriner 1987; Martin and Cailliet 1988). The application of length–frequency analysis is, however, generally restricted to discerning the discrete modes of the younger year-classes in a population as subsequent modes become progressively more difficult to resolve and interpret as cohorts increase in age (Fitz and Daiber 1963; Brander and Palmer 1985; Abdel-Aziz 1992).

Other approaches used to determine age in elasmobranchs have included radiometric dating, eye lens weight, tooth replacement rates, and the vertebral accumulation of mercury. The application of these techniques has, however, met with limited success (Strasburg 1963; Applegate 1965; Moss 1977; Ketchen 1975; Welden et al. 1987; Siezen 1989).

The comparison of age estimates between different calcified structures in teleost fish has been routinely employed as a procedure to verify estimates of age (Beamish and McFarlane 1987). In large oceanic pelagic fish (such as tuna and billfishes), otoliths, scales, finrays, vertebrae, sagittae, and operculae have been used successfully for this purpose (Casselman 1983).

To date, the comparison of age estimates derived from different hard parts has not been applied successfully to the assessment of age in elasmobranchs. This paper addresses this deficit and investigates the comparative use of vertebral centra and caudal thorns in the determination of age estimates for four commercially important skate species. A commercial fishery for skates and rays began around the Falkland Islands in 1991. The fishery is multispecies in composition and has realised between 3500 and 8500 t (unprocessed weight) per annum between 1991 and 1997 (Falkland Islands Government 1998). Little biological information is available on the species targeted and the management approach to this fishery has been conservative as a result.

Materials and methods

Collection methodology

A total of 104 *Bathyraja brachyurops*, 126 *Bathyraja griseocauda*, 47 *Bathyraja scaphiops*, and 51 *Bathyraja albomaculata* were obtained by scientific fisheries observers from commercial fishing vessels and scientific research cruises within the Falkland Islands Interim Conservation and Management Zone between 1994 and 1996 (Falkland Islands: 51.45°S, 59.30°W). Individuals were selected from the size range of each species. An optimum of five specimens from each 5-cm size-class by sex formed the sampling objective (Fig. 1).

A vertebral segment containing the first 10 vertebrae immediately posterior to the scapular origin of the vertebral column was extracted and frozen. The first five caudal thorns, posterior to the first haemal arch of the vertebral column (Hubbs and Ishiyama 1968), were also removed from each specimen. A slice was made from posterior to anterior that bisected the caudal vertebral column horizontally and in so doing removed a segment of the tail containing the required thorns.

Tissue was removed from both the vertebral and thorn segments by immersing the segments in hot water. Thorns were removed from surrounding tissue using forceps and cleaned gently in running water prior to drying. Vertebral segments and extracted thorns were subsequently air dried for storage prior to further processing.

Preparation and processing techniques

Vertebral centra

Vertebral sections were immersed in a 5% solution of trypsin for 24 h to remove the neural and haemal arches and the intervertebral connective tissue. The two largest centra from the thoracic region of each vertebral column were selected for preparation, as the internal pattern of vertebrae is generally more difficult to read with decreasing size (Taylor and Holden 1964; Cailliet et al. 1983*b*; Officer et al. 1996). The centra were sectioned longitudinally with a bench grinder and polished by hand with progressively finer grades of carborundum paper (P620–P1200) using tap water as a lubricant. The polished faces of the centra were embedded in epoxy resin and were further ground and polished to a section thickness of 60–90 μm. Very small, fragile centra (<8 mm centrum diameter) were embedded whole in epoxy resin before grinding.

Previously successful enhancement techniques for elasmobranch centra were assessed for each of the species examined (e.g., alizarin red (LaMarca 1966), silver nitrate (Stevens 1975), cobalt nitrate (Hoenig and Brown 1988)). The most effective methodology proved to be a modification of a grinding and polishing technique described by Smith (1984) followed by staining.

Centrum segments were etched in a dilute solution of acid alcohol (5%) to enhance the band pattern and subsequently immersed for 1–4 h, depending on the species, in a crystal violet solution (0.005%) (Schwartz 1983). The 0.005% solution was used instead of the standard 0.01% solution (Schwartz 1983), as it was found that a longer immersion time in a more dilute solution increased band resolution. Staining times were not dependent on centrum size for *B. albomaculata* and *B. scaphiops*. Centra from these species were stained for 140 min. For *B. brachyurops* and *B. griseocauda*, staining times were increased with increasing centrum size. The optimum staining times were 120 and 70 min, respectively, for centra from these species.

Caudal thorns

Caudal thorns were cleaned by immersion in a 5% solution of trypsin for 12 h. After cleaning, the thorns appeared slightly translucent, with an external band pattern apparent on the thorn surface of all species examined.

The clarity of the banding pattern was improved after etching the thorns in a 5% solution of ethylenediaminetetraacetic acid for periods of between 5 and 10 min. Further enhancement of the band pattern was accomplished by staining the etched thorns. Several staining techniques were assessed (e.g., alizarin red, cobalt nitrate). A modification of a staining technique using silver nitrate (Stevens 1975) proved to be most effective. Thorns were immersed in a 1% solution of silver nitrate for a period of 40–70 min, which was followed by exposure to ultraviolet light.

This technique involves the substitution of calcium in calcium salts in the matrix of the thorn by silver, which is subsequently reduced to black metallic silver. The reaction was terminated by washing in distilled water for 30 s followed by immersion for 10 min in a 5% solution of sodium thiosulphate. The process enhanced the resolution of calcified bands on the thorns, with these areas appearing as dark ridges on the external surface of an opaque thorn matrix.

Reading methodology

Banding patterns on whole thorns and centrum sections were read using a stereomicroscope at a magnification between 6.7 and 40×. A fibreoptic light source was used to illuminate the vertebrae and thorns dorsally and laterally, which enabled resolution of bands using both transmitted and reflected light.

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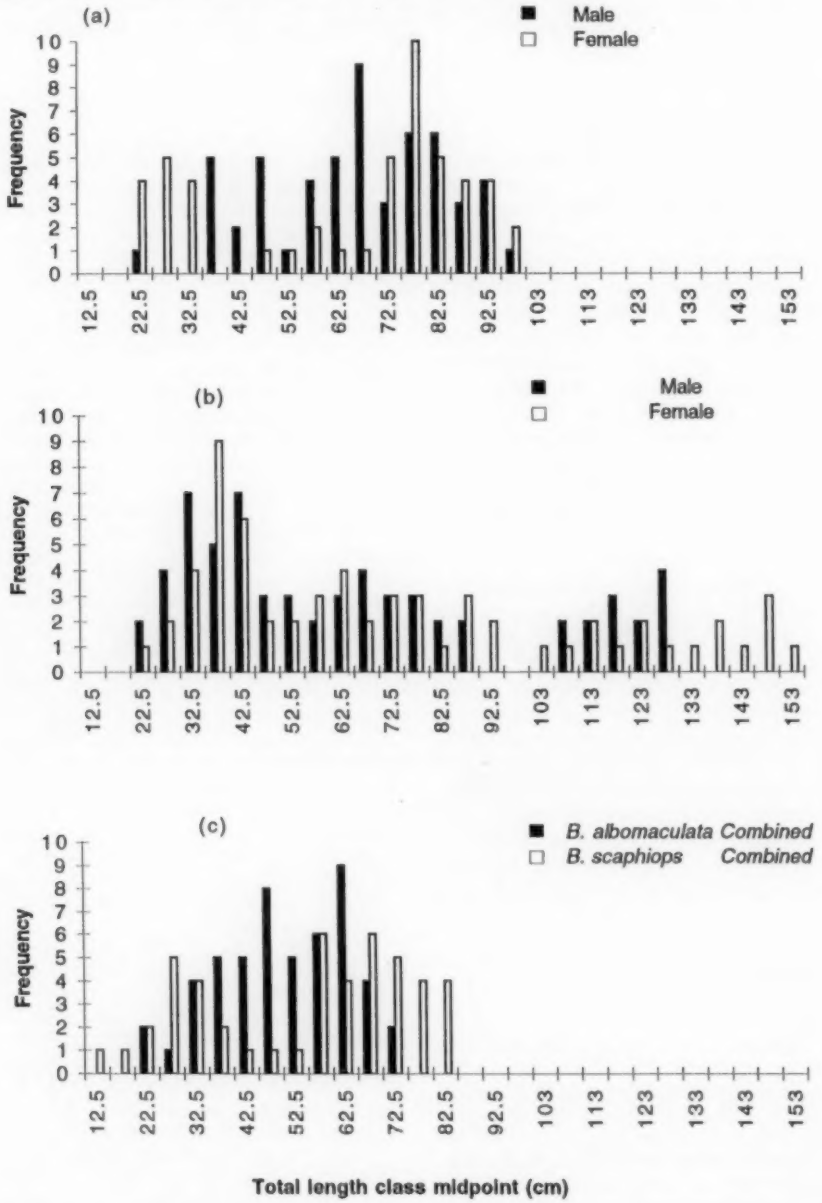
Fig. 1. Length–frequency distribution by sex of specimens of (*a*) *B. brachyurops*, (*b*) *B. griseocauda*, and (*c*) *B. albomaculata* and *B. scaphiops* from which vertebrae and thorns were extracted and processed in this study.



In centrum sections, the axis of the centra showing a complete and distinct series of stained bands was chosen as the main reading axis. Bands were counted from the centre of the section to the distal margin along this axis. The three remaining axes on each section were used during the reading process to verify band occurrences on the main axis and provide corroboration for band counts in areas of poor resolution on the primary axis.

Banding patterns on caudal thorns were read with reference to a protothorn or embryonic thorn that was evident on the apex of all thorns examined (Fig. 2). The base of the protothorn was used as the datum from which all other subsequent bands on the anterior surface were counted. Each of the prepared centra and thorns were read on three independent occasions by one reader in a double-blind study. A subsample of thorns (32 from *B. brachyurops*, 35 from *B. griseocauda*, 24 from *B. scaphiops*, and 26 from *B. albomaculata*) and centra (37 from *B. brachyurops*, 33 from *B. griseocauda*, 26 from *B. scaphiops*, and 26 from *B. albomaculata*) was also read by a second and third reader on three separate occasions, independently, for a within- and between-reader comparison.

Thorn and centrum band count precision was assessed on this subsample of centra and thorns from each of the species examined using Chang's (1982) coefficient of variation (*V*) and index of precision (*D*) described as follows:

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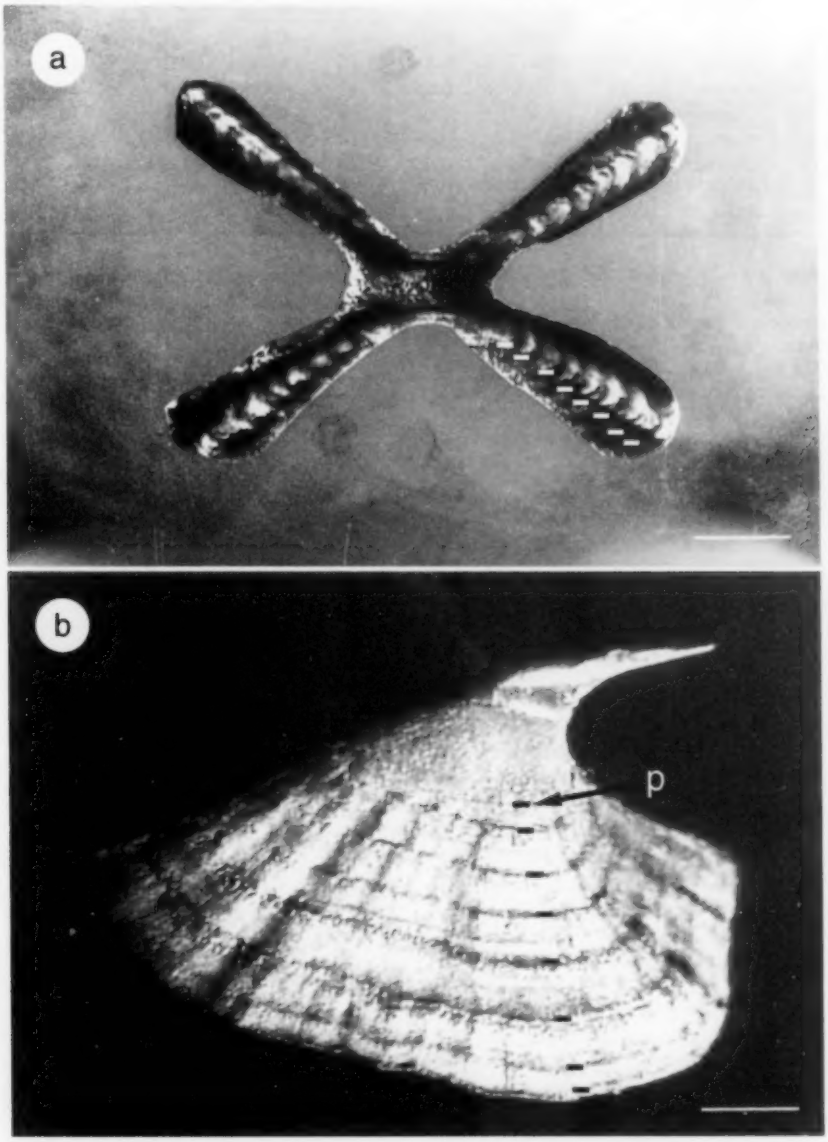
Fig. 2. (*a*) Photomicrograph of a vertebral centrum in transverse section stained with 0.005% crystal violet. Scale bar = 1 mm. (*b*) Photomicrograph of a caudal thorn in lateral view stained with silver nitrate. Scale bar = 1.1 mm; p = protothorn. Both structures were extracted from an immature male *B. griseocauda* of 654 mm total length, estimated to be 7 years old. Annual increments are indicated by small bars on each structure (white bars on Fig. 2*a* and black on Fig. 2*b*).



$$V = \frac{s}{\bar{Y}} \times 100$$

$$D = \frac{\left(\frac{s}{\bar{Y}} \times 100\right)}{\sqrt{R}}$$

where s is the standard deviation of repeated counts, R is the number of counts per individual, and $\bar{Y}$ is the mean of repeated counts. The index of precision represents the percent error contributed by each reading to the average of multiple readings of the same structure.

The similarity of thorn and centrum band counts for each of the specimens processed by the primary reader was examined using a paired t test, while the correlation between counts was assessed using Pearson's product-moment correlation. Male and female *B. brachyurops* and *B. griseocauda* were treated separately, while the sexes were combined for *B. scaphiops* and *B. albomaculata* due to their small sample sizes.

Centrum and thorn edge analysis

Periodicity of band formation on the surface of thorns and within the structure of the vertebral centrum was assessed by correlating the date of capture with the position of the peripheral band

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Table 1. Chang's (1982) coefficient of variation ($\overline{V}$) and mean reader precision ($\overline{D}$) for band counts made by multiple readers on samples of vertebral centra and caudal thorns from *B. brachyurops*, *B. griseocauda*, *B. albomaculata*, and *B. scaphiops*.

		Primary reader			Reader 2			Reader 3		
Species	Structure	N	$\overline{V}$	$\overline{D}$	N	$\overline{V}$	$\overline{D}$	N	$\overline{V}$	$\overline{D}$
B. brachyurops	Centra	37	0.055	0.032	37	0.087	0.050	37	0.107	0.062
	Thorns	32	0.041	0.024	32	0.084	0.049	32	0.088	0.051
B. griseocauda	Centra	33	0.049	0.029	33	0.119	0.069	33	0.155	0.090
	Thorns	35	0.067	0.039	35	0.099	0.057	35	0.121	0.070
B. albomaculata	Centra	26	0.069	0.040	26	0.110	0.064	26	0.113	0.065
	Thorns	24	0.056	0.032	24	0.093	0.054	24	0.086	0.050
B. scaphiops	Centra	26	0.088	0.051	26	0.097	0.056	26	0.088	0.051
	Thorns	26	0.092	0.053	26	0.086	0.049	26	0.106	0.061

forming subsequent to the last fully formed band on a selection of samples from each species.

The band structure evident in the distal edge zone of each of the structures was categorised using a modification of the grading system described by Yudin and Cailliet (1990). The grades were as follows. (1) Centrum: opaque band forming at the marginal edge; thorn: narrow ridge forming at the marginal edge; (2) centrum: translucent band beginning to form at the marginal edge; thorn: broad band beginning to form at the marginal edge; (3) centrum: translucent band well formed at the marginal edge; thorn: broad band well formed at the marginal edge.

Mark–recapture

In support of this study, a programme of mark–recapture involving intraperitoneal injection of oxytetracycline (25 mg·kg body mass^{-1}) has accounted for 550 bathyrajids being tagged to date. This approach has proven to be successful in the validation of several species of elasmobranchs (e.g., Gruber and Stout 1983; Ryland and Ajayi 1984; Smith 1984; Casey et al. 1985; Tucker 1985; Branstetter 1987).

Results

Of the conventional staining techniques previously used on elasmobranch vertebral centra, staining with silver nitrate was the only technique to significantly enhance the banding pattern on thorns from the species examined. The process proved to be very effective and consistent on the thorns of all species and was particularly quick and efficient in its application.

The precision, consistency, and repeatability of thorn and centra counts revealed no significant differences within readers when tested using Chang's (1982) coefficient of variation and index of precision (Table 1).

Differences in precision among readers were apparent, however, when the counts from samples of equal size were compared. With the exception of *B. scaphiops*, there was greater variation and less precision in the centra and thorn count estimates made by the second and third readers than those made by the more experienced primary reader. Thorn band count precision was generally of the same magnitude as that for centra within species. In general, only small differences in precision were recorded between structures by each reader. In the majority of cases, however, the precision of thorn band counts was greater than that of counts from vertebral centra.

Comparison of individual thorn and centra counts by sex revealed similar differences related to structure. All groups tested exhibited a high product-moment correlation (>0.85), implying the existence of a relatively consistent relationship between thorn and centra counts (Table 2). Differences between the sexes were seen between counts in *B. brachyurops* and *B. griseocauda*. In both species, there was greater agreement between counts in females, although this was not significant in either case.

Figure 3 presents the distribution of count differences for the primary reader. Centra and thorn counts agreed exactly in 129 (39%) of 328 measurements made by the primary reader over the entire range of samples examined. Centra counts were greater than thorn counts in 18% (58) of cases and less than thorn counts in 26% (85) of cases. In all species except *B. brachyurops*, the distribution of count differences was skewed, with centra counts being lower than thorn counts. Male *B. brachyurops* and female *B. griseocauda* showed the greatest difference in this regard.

Edge analysis of thorns and centra in all species showed similar patterns of calcification in temporally similar samples. Although variability is apparent in the timing of band formation between species and structures, a trend in band formation can be established. At the end of the autumn and entering winter (June), the grade 3 phase dominated the edge characteristics of the majority of samples (Fig. 4). Opaque band formation (grade 1) was also apparent in this month, implying that June and July are most likely to be the changeover period from translucent to opaque band formation in vertebrae and from broad band to narrow ridge deposition in thorns. The grade 1 phase was prevalent in samples of vertebrae from *B. brachyurops* collected during the spring (September). For samples collected entering the summer months (October), the grade 1 phase was also apparent. November is the probable period of changeover from grade 1 to grade 2. This would appear to occur at a slightly earlier stage in thorns than in vertebrae, as grade 1 appeared to dominate in vertebrae and grade 2 dominated in thorns collected during this month. An annual cycle of translucent and opaque bands in centra and a ridge-defined growth cycle in caudal thorns are therefore supported by these observations.

A single tagged and tetracycline-marked specimen of a related bathyrajid species was recovered. The animal was an immature male and was at liberty for 10 months (tagged 8 July 1994, recaptured 6 May 1995). This species is morphologically similar to and a cohabitor with *B. brachyurops* and consequently is often misidentified as *B. brachyurops*. A de-

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Table 2. Comparison of the caudal thorn and vertebral centrum band counts between the sexes in *B.brachyurops* and *B. griseocauda*, and combined by sex in *B. albomaculata* and *B. scaphiops*.

Species	Count type	df	*N*	Mean	Variance	*t*	Mean of paired difference	*P*	R^2
B. brachyurops									
Male	Thorn	54	55	8.946	8.867	0.866	0.127	0.390	0.886
	Vertebral	54	55	9.073	10.402				
Female	Thorn	48	49	8.449	18.919	–0.125	–0.020	0.901	0.934
	Vertebral	48	49	8.429	19.833				
B. griseocauda									
Male	Thorn	62	63	6.142	13.641	–4.073	–0.397	<0.0001	0.965
	Vertebral	62	63	5.746	15.773				
Female	Thorn	62	63	6.683	15.833	–1.206	–0.206	0.232	0.909
	Vertebral	62	63	6.476	19.737				
B. albomaculata (combined sexes)	Thorn	50	51	8.64	12.953	–0.922	–0.177	0.361	0.862
	Vertebral	50	51	8.471	13.134				
B. scaphiops (combined sexes)	Thorn	46	47	6.617	12.894	–0.375	–0.064	0.710	0.899
	Vertebral	46	47	6.553	13.427				

Note: Student's paired *t* statistic with associated probablity values (*P*) are given; Pearson's product-moment correlation (R^2) values, indicating the correlation between thorn and vertebral bands, are also presented.

scription of this species is currently being prepared by the second author.

Examination of unstained centrum sections and caudal thorns from this winter-tagged animal under ultraviolet light revealed a distinct fluorescent mark on both structures. On centrum sections and in thorns, growth was apparent distal to the position of the tetracycline mark. A variety of staining techniques were used on centrum sections (crystal violet, alizarin red, and silver nitrate) that demonstrated that tetracycline was incorporated into a calcium-dense opaque band at this time of year. The formation of a translucent band distal to the heavily stained band was evident. The centrum edge was thus characterised as approaching grade 3, which corroborates the evidence from the centrum edge study conducted on each of the species.

Tetracycline was incorporated in a narrow ridge in the thorns, suggesting that ridge formation occurs during the winter. A broad band was apparent distal to the fluorescent mark and the edge categorised as approaching grade 3. Although this recaptured specimen was not one of the species investigated in this study, analysis of the stained vertebral sections and caudal thorns revealed that the animal had eight bands at tagging (the assumed point of tetracycline incorporation). This agrees with the size at age for *B. brachyurops*, and therefore, it is assumed that band formation occurs at a similar rate in this recaptured species as in the other species investigated in this study.

Discussion

To date, the hard parts used to estimate age in elasmobranchs have been restricted to vertebral centra and dorsal fin spines (Correia and Figueiredo 1997). Several studies have effectively used spines in age determination studies. However, comparison of age estimates from spines (e.g., Holden and Meadows 1962; Ketchen 1975) with readings from centra has generally proved inconclusive, due to the incomplete and irregular banding pattern observed in the vertebrae (Kaganovskaia 1933; Soldat 1982; Cailliet et al. 1983*b*; Polat and Gumus 1995). Lack of such agreement is generally accepted and has been highlighted in a number of previous ageing studies using hard parts in bony fish (Casselman 1983; Beamish and McFarlane 1987).

Although of similar defensive purpose and placoid scale origin, the structural differences between elasmobranch dorsal fin spines and the caudal thorns of the rajids are significant. In contrast with the enamel-like structure of fin spines, the caudal thorns of the bathyrajids examined in this study are convexly placoid and securely embedded and anchored within the caudal tissue. The thorns exhibit a definite external sculpture with distinctive circular ridges that vary in definition and resolution among species. The apparent periodicity and definition of the surface sculpture in the species lead to the investigation of these structures as tools for the determination of age. Initial observations reveal that thorn growth and band formation occurs in a manner similar to that of dorsal spines in *Squalus acanthias* (Holden and Meadows 1962). This process is currently being investigated through histological analysis by the senior author.

Thorns have a number of distinct advantages over centra in their use as potential ageing tools. Thorns can be removed and cleaned with comparative ease and, importantly, may be removed unobtrusively and with minimal damage to fish of commercial value. A major advantage with thorns is that they can also be removed without sacrifice of or detrimental effect to the animal, a feature that is likely to prove invaluable in tagging studies where species suiting the technique are the focus of research. Difficulties have arisen in previous studies in the determination of the counting reference point or nucleus and the first growth band (e.g., Brander and Palmer 1985) in centra used as ageing tools. The caudal thorns of the species examined in this study showed that location of the first growth band is perhaps the most distinctive of all the external sculpture and is recognised with relative ease, even prior to staining. Given that previous studies have met with varied levels of success comparing readings

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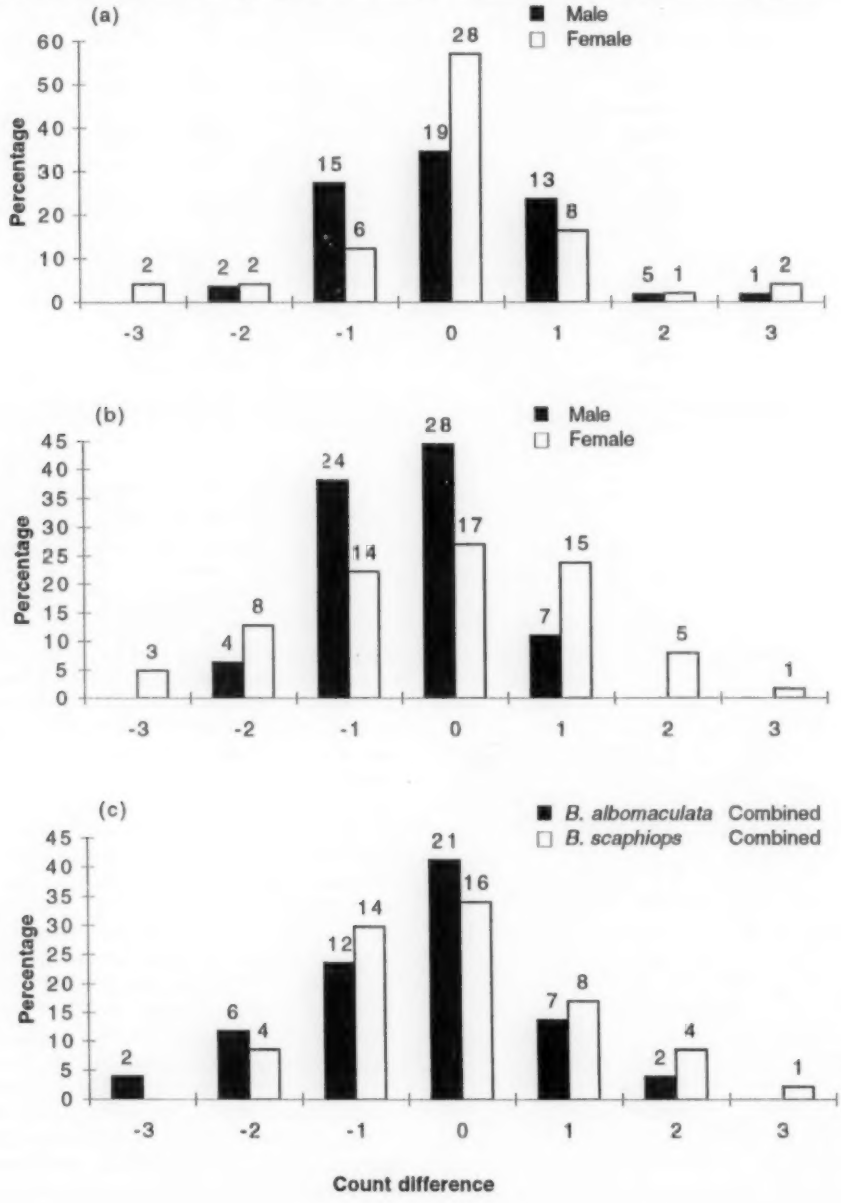
Fig. 3. Percent frequency, by sex, of agreement between vertebral centrum and thorn band counts in (*a*) *B. brachyurops*, (*b*) *B. griseocauda*, and (*c*) *B. scaphiops* and *B. albomaculata* made by the primary reader. Column totals indicate number of individuals. The difference between counts is expressed as the vertebral centrum count less the thorn band count.



from hard parts and vertebrae, the high level of agreement existing between thorn and centrum band counts in the species examined in this study is encouraging.

Although reader precision appears to be related to thorn morphology and size, the ease with which thorns can be read by readers with little experience, to a high degree of precision, advocates the use of thorns, where possible, in preference to vertebral centra as ageing structures.

In this study, reader precision was highest for thorn band counts from species with large thorns carrying a distinct banding pattern (e.g., *B. albomaculata*). Similarly, the lowest level of precision was associated with small thorns and difficulties experienced resolving thorn banding pattern due to poor and indistinct surface sculpture (e.g., *B. scaphiops*).

The consistent disagreement between counts in male *B. griseocauda* and to some extent in male *B. brachyurops* over the size range examined suggests a possible physiological difference effecting the calcification of the vertebral centra, which may be associated with dietary or temperature changes throughout life (Jones and Geen 1977; Ferreira and

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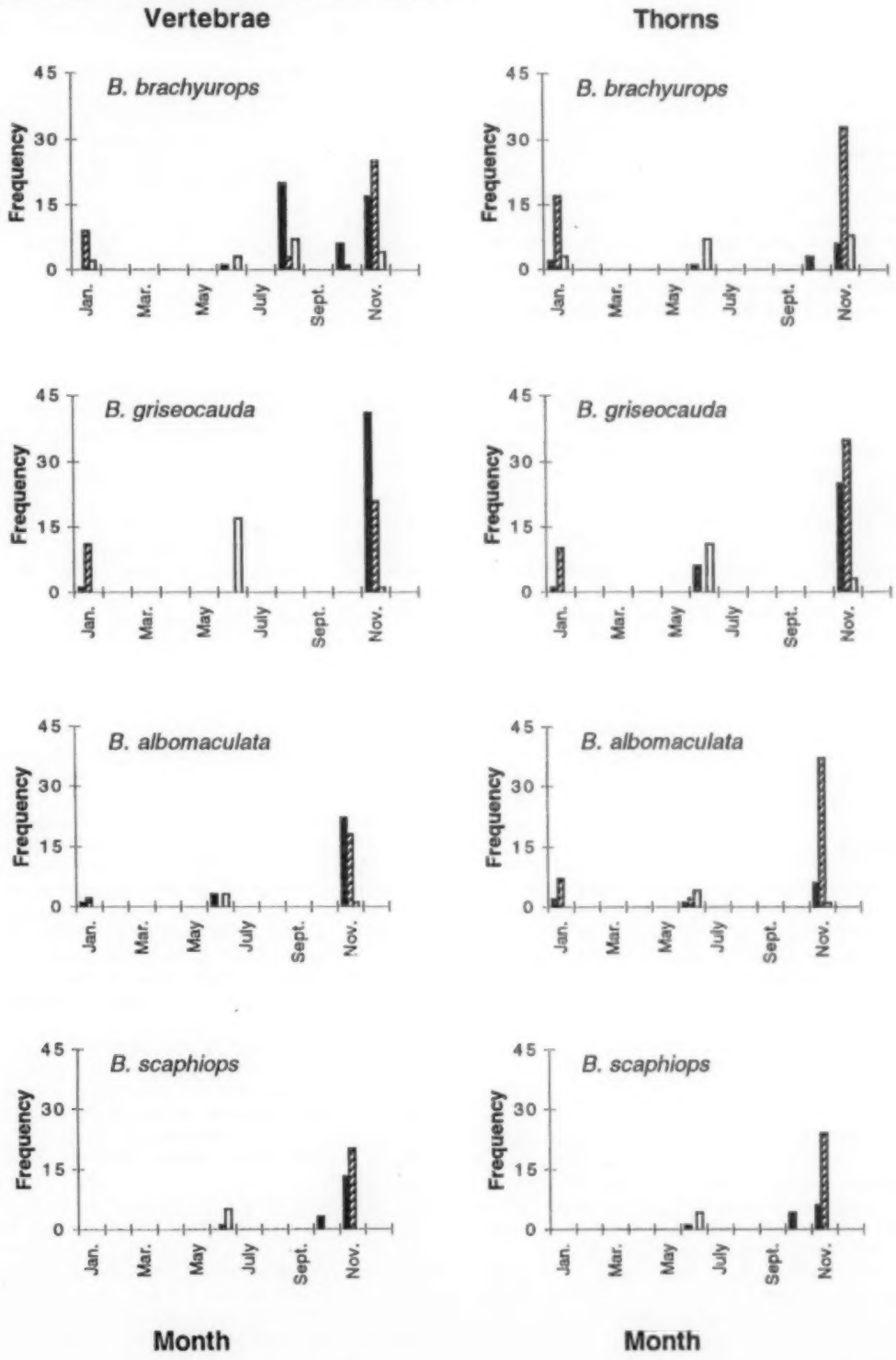
Fig. 4. Temporal characteristics in the structure of the growing edge of vertebral centra and thorns from *B. brachyurops*, *B. griseocauda*, *B. albomaculata*, and *B. scaphiops* described using a modification of the grading system defined by Yudin and Cailliet (1990). Solid bars, grade 1; hatched bars, grade 2; open bars, grade 3.



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Vooren 1991). The banding pattern in vertebral sections in both sexes of *B. griseocauda* appears to alter in density and variability with increasing body size. This leads to difficulties in interpreting and resolving bands, a situation that has previously been encountered in other elasmobranch species (e.g., Stevens 1975; Casey et al. 1985; Ferreira and Vooren 1991; Officer et al. 1997).

The propensity for underreading of the banding pattern in centra, relative to thorn band counts, particularly in male individuals, suggests poor resolution of periodic structures within centrum sections that is likely to be most prevalent in the centra of older, slower growing individuals, particularly at the centre and distal margins.

Thorn and centrum edge analysis describes a structural cycle of growth band formation that supports the supposition of annual periodicity. Analysis of the vertebral centra and thorns from a tetracycline-marked and recovered specimen lends support to the conclusions of the centrum edge analysis that a single band pair is laid down annually in the vertebrae of the species investigated in this study. Similarly, the position of the fluorescent mark relative to the date of tagging indicates that tetracycline was incorporated into an opaque, calcium-rich band in vertebral sections. This agrees with previous findings in other elasmobranch species where hypermineralisation of vertebrae occurs when growth is slowest (Ishiyama 1951; Yudin and Cailliet 1990; Officer et al. 1997). Both thorn edge analysis and the position of the fluorescent mark reveal that narrow ridge formation occurs during the slower growth phase in caudal thorns. It is hoped that with further recaptures of tetracycline-marked specimens that validation at the species level will be achieved.

The novel approach to estimating age in skates and rays using caudal thorns is a quick, easy, and reliable method. Given the accepted difficulties dedicating resources to the conventional vertebral ageing of these species, the application of this technique, where appropriate, has the potential to be of significant benefit to the resource assessment and management of skate and ray stocks.

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References

Abdel-Aziz, S.H. 1992. The use of vertebral rings of the brown ray *Raja miraletus* (Linnaeus, 1758) off Egyptian Mediterranean coast for estimation of age and growth. Cybium, **16**: 121–132.

Applegate, S.P. 1965. Tooth terminology and variation in sharks with special reference to the sand shark, *Carcharhinus taurus* Rafinesque. Los Angel. Cty. Mus. Contrib. Sci. **85**.

Beamish, R.J., and McFarlane, G.A. 1987. Current trends in age determination methodology. *In* Age and growth of fish. *Edited by* R.C. Summerfelt and G.E. Hall. Iowa State University Press, Ames, Iowa. pp. 15–42.

Brander, K., and Palmer, D. 1985. Growth rate of *Raja clavata* in the northeast Irish Sea. J. Cons. Int. Explor. Mer, **42**: 125–128.

Branstetter, S. 1987. Age and growth validation of newborn sharks held in laboratory aquaria, with comments on the life history of the Atlantic sharpnose shark, *Rhizoprionodon terraenovae*. Copeia, **1987**: 291–300.

Cailliet, G.M., Martin, L.K., Kusher, D., Wolf, P., and Weldon, B.A. 1983*a*. Techniques for enhancing vertebral bands in age estimation of California elasmobranchs. *In* Proceedings of the International Workshop on Age Determination of Oceanic Pelagic Fishes: Tunas, Billfishes and Sharks. *Edited by* E.D. Prince and L.M. Pulos. NOAA Tech. Rep NMFS No. 8. pp. 157–165.

Cailliet, G.M., Martin, L.K., Harvey, J.T., Kusher, D., and Weldon, B.A. 1983*b*. Preliminary studies on the age and growth of blue, *Prionace glauca*, common thresher, *Alopius vulpinus*, and shortfin mako, *Isurus oxyrinchus*, sharks from California waters. *In* Proceedings of the International Workshop on Age Determination of Oceanic Pelagic Fishes: Tunas, Billfishes and Sharks. *Edited by* E.D. Prince and L.M. Pulos. NOAA Tech. Rep. NMFS No. 8. pp. 179–188.

Casey, J.G., Pratt, H.L., Jr., and Stillwell, C.E. 1985. Age and growth of the sandbar shark (*Carcharinus plumbeus*) from the western North Atlantic. Can. J. Fish. Aquat. Sci. **42**: 963–975.

Casselman, J.M. 1983. Age and growth assessment of fish from their calcified structures — techniques and tools. *In* Proceedings of the International Workshop on Age Determination of Oceanic Pelagic Fishes: Tunas, Billfishes and Sharks. *Edited by* E.D. Prince and L.M. Pulos. NOAA Tech. Rep. NMFS No. 8. pp. 1–17.

Chang, W.Y.B. 1982. A statistical method for evaluating reproducibility of age determination. Can. J. Fish. Aquat. Sci. **39**: 1208–1210.

Correia, J.P., and Figueiredo, I.M. 1997. A modified decalcification technique for enhancing growth bands in deep-coned vertebrae of elasmobranchs. Environ. Biol. Fishes, **50**: 225–230.

Daiber, F.C. 1960. A technique for age determination in the skate, *Raja eglanteria*. Copeia, **1960**: 258–260.

Du Buit, M.H. 1972. Age et croissance de *Raja batis* et de *Raja naevus* en Mer Celtique. J. Cons. Cons. Int. Explor. Mer, **37**: 261–265.

Falkland Islands Government. 1998. Fisheries Department, fisheries statistics. Vol. 2. Falkland Islands Government Printing Office, Stanley, Falkland Islands.

Ferreira, B.P., and Vooren, C.M. 1991. Age, growth, and structure of vertebrae in the school shark *Galeorhinus galeus* (Linnaeus, 1758) from southern Brazil. Fish. Bull. U.S. **89**: 19–31

Fitz, E.S., Jr., and Daiber, F.C. 1963. An introduction to the biology of *Raja eglanteria* (Bosc, 1802) and *Raja erinacea* (Mitchell, 1825) as they occur in Delaware Bay. Bull. Bingham Oceanogr. Collect. Yale Univ. **18**: 69–97.

Gruber, S.H., and Stout, R.G. 1983. Biological materials for the study of age and growth in a tropical marine elasmobranch, the lemon shark, *Negaprion brevirostris* (Poey). *In* Proceedings of the International Workshop on Age Determination of Oceanic Pelagic Fishes: Tunas, Billfishes and Sharks. *Edited by* E.D. Prince and L.M. Pulos. NOAA Tech. Rep. NMFS No. 8. pp. 193–205.

Hoenig, J.M., and Brown, C.A. 1988. A simple technique for staining growth bands in elasmobranch vertebrae. Bull. Mar. Sci. **42**: 334–337.

Holden, M.J., and Meadows, P.S. 1962. The structure of the spine of the spur dogfish (*Squalus acanthias*) and its use for age determination. J. Mar. Biol. Assoc. U.K. **42**: 179–197.

Holden, M.J., and Vince, M.R. 1973. Age validation studies on the

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centra of *Raja clavata* using tetracycline. J. Cons. Int. Explor. Mer, **35**: 13–17.

Hubbs, C.L., and Ishiyama, R. 1968. Methods for the taxonomic study and description of skates (Rajidae). Copeia, **1968**: 82–91.

Ishiyama, R. 1951. Studies on rays and skates belonging to the family Rajidae, found in Japan and adjacent regions. 2. On the age determination of Japanese black skate *Raja fusca*. Garman (preliminary report). Bull. Jpn. Soc. Sci. Fish. **16**: 112–118.

Ishiyama, R. 1958. Studies on the rajid fishes found in the waters around Japan. Shim. Coll. Fish. **7**: 1–202.

Jones, B.C., and Geen, G.H. 1977. Age determination of an elasmobranch (*Squalus acanthias*) by X-ray spectrometry. J. Fish. Res. Board Can. **34**: 44–48.

Kaganovskaia, S.M. 1933. A method of determining the age and the composition of the catches of the spiny dogfish (*Squalus acanthias*). Bull. Far East Acad. Sci. U.S.S.R. **1–3**: 139–141. (Transl. from Russian. Fish. Mar. Serv. Transl. Ser. No. 281, 1960)

Ketchen, K.S. 1975. Age and growth of the dogfish *Squalus acanthias* in British Columbia waters. J. Fish. Res. Board Can. **32**: 43–59.

LaMarca, M.J. 1966. A simple technique for demonstrating calcified annuli in the vertebrae of large elasmobranchs. Copeia, **1966**: 351–352.

Martin, L.K., and Cailliet, G.M. 1988. Age and growth determination of the bat ray *Myliobatis californica* in central California. Copeia, **1988**: 762–773.

Moss, M. 1977. Skeletal tissues in sharks. Am. Zool. **17**: 335–342.

Natanson, L.J. 1993. Effect of temperature on band deposition in the little skate *Raja erinacea*. Copeia, **1993**: 199–206.

Officer, R.A., Gason, A.S., Walker, T., and Clement, J.G. 1996. Sources of variation in counts of growth increments in vertebrae from gummy shark, *Mustelus antarcticus*, and school shark, *Galeorhinus galeus*: implications for age determination. Can. J. Fish. Aquat. Sci. **53**: 1765–1777.

Officer, R.A., Day, R.W., Clement, J.G., and Brown, L.P. 1997. Captive gummy sharks, *Mustelus antarcticus*, form hypermineralized bands in their vertebrae during winter. Can. J. Fish Aquat. Sci. **54**: 2677–2683.

Polat, N., and Gumus, A. 1995. Age determination of the spiny dogfish (*Squalus acanthias*) in Black Sea waters. Isr. J. Aquacult. Bamidgeh, **47**: 117–124.

Ryland, J.S., and Ajayi, T.O. 1984. Growth and population dynamics of three *Raja* species (Batoidei) in Carmarthen Bay, British Isles. J. Cons. Int. Explor. Mer, **41**: 111–120.

Schwartz, F.J. 1983. Shark ageing methods, and age estimation of scalloped hammerhead, *Sphyrna lewini*, and dusky, *Carcharinus obscurus*, sharks based on vertebral ring counts. *In* Proceedings of the International Workshop on Age Determination of Oceanic Pelagic Fishes: Tunas, Billfishes and Sharks. *Edited by* E.D. Prince and L.M. Pulos. NOAA Tech. Rep. NMFS No. 8. pp. 167–174.

Siezen, R.J. 1989. Eye lens aging in the spiny dogfish (*Squalus acanthias*). I. Age determination from lens weight. Curr. Eye Res. **8**: 707–712.

Smith, J.W., and Merriner, J.V. 1987. Age and growth, movements and distribution of the cownose ray, *Rhinoptera bonasus*, in Chesapeake Bay. Estuaries, **10**: 153–154.

Smith, S.E. 1984. Timing of vertebral-band deposition in tetracycline injected leopard sharks. Trans. Am. Fish. Soc. **113**: 308–313.

Soldat, V.T. 1982. Age and size of spiny dogfish, *Squalus acanthias*, in the Northwest Atlantic. NAFO Sci. Counc. Stud. **3**: 47–52.

Steven, G.A. 1936. Migration and growth of the thornback ray (*Raia clavata* L.). J. Mar. Biol. Assoc. U.K. **20**: 605–614.

Stevens, J.D. 1975. Vertebral rings as a means of age determination in blue shark (*Prionace glauca* L.). J. Mar. Biol. Assoc. U.K. **55**: 657–665.

Strasburg, D.W. 1963. The diet and dentition of *Isistius brasiliensis*, with remarks on tooth replacement in other sharks. Copeia, **1963**: 33–40.

Taylor, A.J., and Holden, M.J. 1964. The preparation and use of vertebrae for age determination in rays. ICES CM Near North Seas Comm. **145**: 1–4.

Tucker, R. 1985. Age validation studies on the spines of the spurdog (*Squalus acanthias*) using tetracycline. J. Mar. Biol. Assoc. U.K. **65**: 641–651.

Waring, G.T. 1984. Age, growth and mortality of the little skate off the northeast coast of the United States. Trans. Am. Fish. Soc. **113**: 314–321.

Welden, B.A., Cailliet, G.M., and Flegal, A.R. 1987. Comparisons of radiometric with vertebral band age estimates in four California elasmobranchs. *In* Age and growth of fish. *Edited by* R.C. Summerfelt and G.E. Hall. Iowa State University Press, Ames, Iowa. pp. 301–315.

Younger, W.D. 1975. Age and growth estimation in population size. *In* Principles of fishery science. *Edited by* W.H. Everhart, A.W. Eipper, and W.D. Young. Iowa State University Press, Ames, Iowa. pp. 301–315.

Yudin, K.G., and Cailliet, G.M. 1990. Age and growth of the gray smoothound, *Mustelus californicus*, and the brown smoothound, *M. henlei*, sharks from central California. Copeia, **1990**: 191–204.

Zeiner, S.J., and Wolf, P. 1993. Growth characteristics and estimates of age at maturity of two species of skates (*Raja binoculata* and *Raja rhina*) from Monterey Bay, California. NOAA Tech. Rep. NMFS, **115**: 87–99.

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Influence of salmon spawner densities on stream productivity in Southeast Alaska

Mark S. Wipfli, John P. Hudson, Dominic T. Chaloner, and John P. Caouette

Abstract: We conducted this study to determine the relationship between salmon spawner abundance and stream biofilm and benthic macroinvertebrate abundance in Southeast Alaska. Experiments took place in outdoor artificial and natural streams. Six pink salmon (*Oncorhynchus gorbuscha*) carcass treatments (0.00, 1.45, 2.90, 4.35, 5.80, and 7.25 kg wet mass) placed in artificial channels were subsampled repeatedly for biofilm ash-free dry mass (AFDM), chlorophyll *a*, and macroinvertebrates. In a small (nonanadromous) forest stream, we sampled benthos throughout a 66-m reach 17 days after distributing 60 carcasses along the lower half of that reach. All response variables significantly increased in response to carcass additions in both artificial and natural streams. Chlorophyll *a* continued to increase across all loading rates, while AFDM and total macroinvertebrate densities showed no further response to loading beyond the first treatment (1.45 kg) in artificial streams. In the natural stream, AFDM and chironomid densities continued increasing across loading levels. These results indicated that increased spawner densities increased lower trophic level abundance until a trophic capacity was reached. Salmon escapement goals should consider food web effects, especially on trophic levels that support juvenile salmonids, that ultimately affect freshwater salmon production.

Résumé : Nous avons entrepris cette étude pour déterminer la relation entre, d'une part, l'abondance des saumons reproducteurs et, d'autre part, l'abondance des films biologiques fluviaux et des macroinvertébrés dans le sud-est de l'Alaska. Les expériences ont été effectuées à l'extérieur dans des canaux artificiels et un cours d'eau naturel. On a procédé à cinq traitements consistant en l'introduction de carcasses de saumon rose (*Oncorhynchus gorbuscha*) (1,45, 2,90, 4,35, 5,80, et 7,25 kg poids humide) avec témoin (0,00 kg) dans des canaux artificiels; on y a prélevé à plusiers reprises des sous-échantillons pour mesurer la masse à sec sans cendre des films biologiques ainsi que la chlorophylle *a* et les macroinvertébrés. Dans un petit cours d'eau forestier non fréquenté par des saumons anadromes, nous avons échantillonné le benthos d'un tronçon de 66 m 17 jours après avoir distribué 60 carcasses dans la moitié inférieure de ce tronçon. Toutes les variables mesurées se sont significativement accrues en réponse aux introductions de carcasses tant dans les canaux artificiels que dans le cours d'eau naturel. La quantité de chlorophylle *a* n'a pas cessé de s'accroître d'un traitement à l'autre, tandis que la masse à sec sans cendre des films biologiques et les densités de macroinvertébrés ont plafonné dès le premier traitement (1,45 kg) dans les canaux artificiels. Dans le cours d'eau naturel, la masse à sec sans cendre des films biologiques et les densités des chironomides n'ont cessé de s'accroître d'un traitement à l'autre. Ces résultats ont indiqué que l'accroissement des densités de reproducteurs a eu pour effet d'accroître l'abondance des organismes des niveaux trophiques inférieurs jusqu'à atteinte d'une capacité trophique donnée. Dans l'établissement des objectifs concernant l'échappée des saumons, on devrait considérer les phénomènes intervenant dans le réseau alimentaire, particulièrement ceux qui touchent les niveaux trophiques dont dépendent les salmonidés juvéniles, lesquels déterminent ultimement la production de saumons dans les eaux douces.

[Traduit par la Rédaction]

Introduction

Movements of nutrients, detritus, and prey among habitats can influence populations and community dynamics, increasing consumer resources and subsidizing food webs (Polis et al. 1997). Subsidies across habitats are common in stream ecosystems where they often elevate productivity. For instance, nutrients received from groundwater can stimulate stream primary production (Coleman and Dahm 1990), allochthonous litter from riparian forests comprises the trophic base for detritivores in small forested streams (Cummins et al. 1983), and terrestrial invertebrates falling from riparian vegetation form a large fraction of some salmonid diets (Wipfli 1997).

Contrary to the conventional view that material flows from land to freshwater and then to the sea (Vannote et al. 1980; Maser and Sedell 1994), a massive movement of organic material and nutrients occurs annually from marine to freshwater and terrestrial ecosystems via the migration of anadromous fishes (Levy 1997). This "upstream" marine subsidy to freshwater and land is poorly understood and the ecological effects likely underestimated (Willson et al. 1998). These linkages may be critical for sustaining freshwater and terrestrial productivity, especially at land–water interfaces (Polis et al. 1997; Wipfli et al. 1998).

Alaska and parts of western Canada represent the few re-

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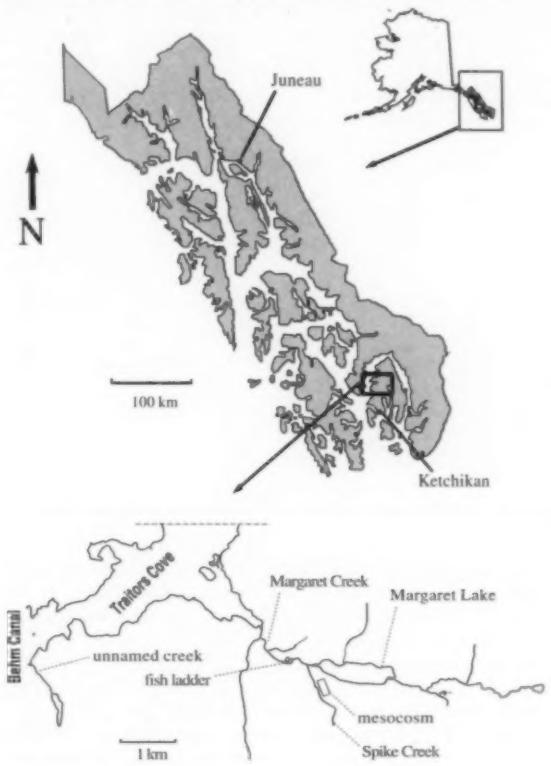
Fig. 1. Study site on Revillagigedo Island, Southeast Alaska.



maining areas of North America that still support historic runs of salmon (Baker et al. 1996; Slaney et al. 1996). Here, millions of salmon return to their natal habitats every year to spawn and die (Wertheimer 1997), bringing with them thousands of tons of marine-derived nutrients (MDN) to freshwater (Mathisen et al. 1988). Southeast Alaska is a major Pacific salmon producer (Wertheimer 1997) and contains over 5200 anadromous salmon streams totaling 40 000 km in length (Halupka et al. 1999). Salmon have great potential to subsidize multiple trophic levels in these systems because (*i*) they are nutrient rich (Mathisen et al. 1988), (*ii*) they contain desirable stoichiometry (Hesson 1997), (*iii*) their runs often span much of the year and achieve high numbers (Groot and Margolis 1991), (*iv*) they can decompose slowly (from weeks to months) (Kline et al. 1997; Wipfli et al. 1998) metering nutrient release over time (Parmenter and Lamarra 1991), and (*v*) they are consumed by many invertebrates and vertebrates in both freshwater and riparian food webs (Cederholm et al. 1989; Bilby et al. 1996; Wipfli et al. 1998).

There are several pathways by which MDN can influence freshwater and riparian food webs. The death and decomposition of salmon after spawning increases freshwater nutrient levels (Mathisen et al. 1988; Schuldt and Hershey 1995); increased productivity associated with this enhanced nutrient supply can influence food webs (Kline et al. 1990; Wipfli et al. 1998). Also, direct material transfer occurs through ingestion of salmon tissue and eggs by invertebrates and fishes including juvenile salmon (Kline et al. 1997; Bilby et al. 1998). This subsidy may be particularly important to juvenile salmon whose overwinter survivorship is largely dependent on body size (Quinn and Peterson 1996), a function of food intake during the growing season (Groot and Margolis 1991). As a consequence of these mechanisms, anadromous salmon runs may not only affect other taxa, but they may also be driving a positive feedback that controls their own productivity and population density.

Stable isotope analyses (carbon and nitrogen) have shown that MDN occur in selected components of freshwater and terrestrial food webs in the Pacific Northwest (Kline et al. 1990; Bilby et al. 1996). It is unclear if MDN are an obligate component of watersheds with salmon runs, truly subsidizing their productivity, or a facultative component where local nutrient supply could otherwise substitute for MDN. In view of the inherent nutrient-limited state of Pacific Northwest freshwater ecosystems (Gregory et al. 1987; Kyle et al. 1997), ecological effects of MDN enrichment could be immense. Further, the ramifications of suppressed or lost salmon runs, and consequent reduced nutrient supply, could be severe. Placing spawned-out hatchery salmon in streams has been considered to help facilitate salmon stock restoration in Washington and Oregon (Levy 1997). Slow-release "salmon bricks" (processed and compressed salmon byproducts designed to meter nutrients into surface waters) as well as artificial fertilizers are also being used as salmon stock restoration tools in Pacific Northwest systems (Ashley and Slaney 1997; P. Kiffney, NMFS, Seattle, Wash., personal communication) where stocks have sharply declined (Gregory and Bisson 1997).

The objectives of this study were to determine the effects of carcass loading (range of salmon carcass densities) on aquatic productivity, in terms of biofilm, chlorophyll *a*, and macroinvertebrate abundance, in artificial and natural streams in Southeast Alaska. Our hypotheses were that (*i*) salmon provide a nutrient subsidy that stimulates freshwater productivity, measured as increased standing stock of lower trophic levels (microbes, invertebrates), and (*ii*) increasing spawner density (i.e., nutrient loading) supports increased lower trophic level standing stock until a trophic capacity is reached beyond which more nutrients provide no additional subsidy. In addition to evaluating an emerging paradigm in ecosystem ecology, marine subsidy of freshwater food webs, these results should aid in developing more complete guidelines for fisheries and ecosystem management, including salmon stock protection and restoration, throughout the North Pacific Rim.

Methods

Study site

This study was conducted on Revillagigedo Island, Southeast Alaska (Fig. 1). Details of the study area, including species of anadromous and resident fishes, are given in Wipfli et al. (1998). Artificial stream experiments spanned from 1 September through 31 October 1997 and a natural stream experiment from 18 August through 4 September 1997.

Mesocosm experiment

The mesocosm experiment was conducted in 30 straight once-through artificial stream channels described by Wipfli et al. (1998), each 250 cm long × 18 cm wide and constructed next to Spike

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Table 1. Salmon carcass treatments and mass and corresponding carcass concentrations and densities used in the mesocosm and natural stream experiments.

	Treatment (mesocosm) or Pool No. (natural stream)					
	1	2	3	4	5	6
Mesocosm						
Number of carcasses[a]	0	1	2	3	4	5
Wet mass (kg)	0	1.45	2.90	4.35	5.80	7.25
Estimated dry mass (kg)	0	0.54	1.07	1.61	2.15	2.68
Carcass concentration per volume of water (kg wet mass·L water^{-1}·s^{-1})	0	3.02	6.04	9.06	12.08	15.10
Carcass mass per streambed area (kg wet mass·m^{-2})	0	3.22	6.44	9.67	12.89	16.11
Natural stream						
Number of carcasses	0	0	0	20	40	60
Cumulative wet mass (kg)	0	0	0	35	73	112
Estimated cumulative dry mass (kg)	0	0	0	13	27	41
Cumulative carcass concentration (kg wet mass·L water^{-1}·s^{-1}) during baseflow (9 L·s^{-1})	0	0	0	4.2	8.7	13.3
Cumulative carcass concentration (kg wet mass·L water^{-1}·s^{-1}) during typical flow (53 L·s^{-1})	0	0	0	0.7	1.4	2.1
Progressive carcass mass per streambed area (kg wet mass·m^{-2}) calculated at baseflow	0	0	0	2.1	1.7	1.9

[a]Minus the tail section from each fish.

Creek, a second-order tributary of Margaret Creek. Stream water from a nonanadromous portion of Spike Creek was gravity-fed with plastic pipes through 170-L sediment traps and then into a header tank. Water flowed from the header tank into each channel through flow-control valves and was then directed back to Spike Creek. The mesocosm was designed to provide unrestricted invertebrate drift with stream flow into and out of channels.

Each channel was divided into three areas: a 60-cm pool for carcass treatments, a 20-cm turbulence reduction area, and a 160-cm riffle. The riffle was subdivided into substrate baskets for sampling invertebrates and tiles for sampling biofilm. Six Vexar® substrate baskets (20 × 18 × 5 cm, 6 × 6 mm opening mesh) were used, each lined with fiberglass mesh (1.6-mm openings) and filled with sand and gravel collected from a natural stream. Eighteen unglazed clay tiles (5 × 5 cm), in a 6 × 3 matrix and level with the substrate baskets, were placed at the downstream end of each riffle. Invertebrates immediately began colonizing substrata in channels via drift from the natural stream. Stream water passed through the channels for 4 days before the experiment began. During the experiment, discharge through channels was 0.48 ± 0.02 L·s^{-1}, and water temperature, continuously logged at 1-h intervals, averaged 8.8°C (range 2.3–12.9°C).

Six treatments were applied: five incremental increases of salmon carcass tissue (1.45, 2.90, 4.35, 5.80, and 7.25 kg wet mass) and a control (no carcass) (Table 1) that fell within the range of spawner densities observed in nearby streams. Live female pink salmon (*Oncorhynchus gorbuscha*) (48–52 cm fork length) with eggs were collected from Margaret Creek on 31 August and 1 September and were given a sharp blow to the head. A portion of the tail and caudal peduncle was removed, such that each fish weighed 1.45 kg (wet mass). Using one gender helped reduce among-channel variability. Although either gender could have been used, we chose females because of the visual abundance of eggs scattered on streambeds during and after spawning, which we postulated might also influence benthic communities. The eggs comprised 15% of fish wet mass. Fish were then randomly selected, and one to five were placed in the pools of channels to produce six treatment levels (Table 1). To determine the mean ratio of dry to wet mass, six fish were weighed wet and then oven dried at 80°C and reweighed. The ratio (0.37) was used to estimate the initial mass of carcass material placed into each channel. Each table was covered with Plexiglas® that was anchored with a wooden frame to protect carcasses from bears and other scavengers. Benthic invertebrates were sampled six times and biofilm was sampled five times during the 60-day experiment. All 30 channels were subsampled during each sampling event.

Benthic macroinvertebrates were sampled from sand–gravel substrata in baskets. Sampling of individual tables was completed each day, but sampling of all tables was spread over 2 days, two tables on the first day and three on the subsequent day. Individual baskets were removed with a 250-μm net placed downstream to collect any dislodged invertebrates. Removed baskets were immediately replaced with another substrate basket. Although this net mesh size may have allowed some small invertebrates to pass through, no treatments or channels were biased; they all received the same collection technique. Macroinvertebrates were washed from substrata, sorted live, identified to the lowest reliable taxon, and counted on-site. Unusual taxa were collected and examined more closely under magnification in the laboratory. Representative specimens are held in the invertebrate collection at the Pacific Northwest Research Station, Juneau, Alaska.

Biofilm was collected from the upper surface of tiles for ash-free dry mass (AFDM) and chlorophyll *a* measurements. Three tiles were removed from each channel and immediately replaced with three new tiles. An estimate was made of surface biofilm area coverage intact after sloughing; biofilm was scraped, brushed, and flushed from each tile with distilled water into a graduated cylinder. The sample was vigorously stirred, split between two cylinders, the total volume noted, and then filtered onto two separate glassfiber filters (Whatman® GF/F); filters used for AFDM were all preashed, prewashed, and preweighed. Samples were kept frozen until they were processed and transported to the laboratory in coolers. Filters for chlorophyll *a* analysis (see Steinman and Lamberti 1996) were stored with dry ice for the shipping to University of Notre Dame. Those for AFDM were oven dried, weighed, ashed at 500°C, and reweighed to determine AFDM (see Steinman and Lamberti 1996).

Natural stream experiment

As in Wipfli et al. (1998), this study focused around a controllable and statistically rigorous mesocosm experiment in conjunction with a natural stream experiment to see whether similar patterns in biota abundance were found in natural and artificial systems. We

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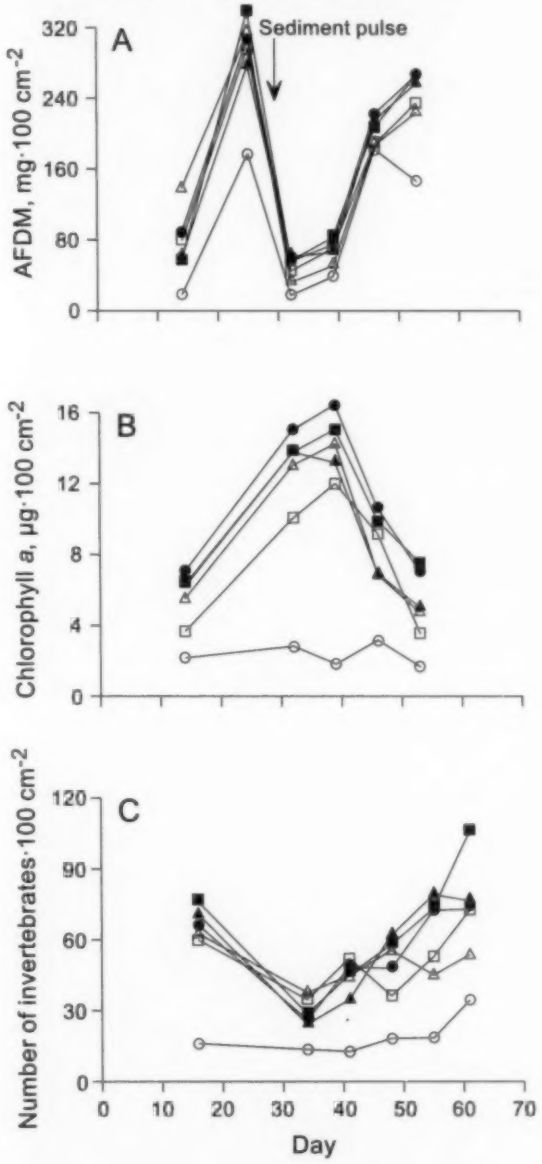
Fig. 2. Mean (A) AFDM, (B) chlorophyll *a* density, and (C) benthic macroinvertebrate density in the mesocosm experiment in response to salmon carcass treatments: 0.00 (○), 1.45 (△), 2.90 (□), 4.35 (●), 5.80 (▲), and 7.25 kg wet mass (■). The arrow represents the timing of a large freshet that caused a sediment pulse to pass through the channels.



used six pool–riffle sequences over a 66-m reach within a non-anadromous portion of a small (2-m-wide) unnamed stream near Margaret Creek. In the riffle below each pool, we placed two 15 × 15 cm unglazed clay tiles and four baskets (25 cm wide × 25 cm long × 10 cm high) filled with natural substrate from Margaret Creek. Tiles were placed on the streambed surface and baskets buried flush with the surface of the rest of the substrata within the riffle. Water temperature in the natural stream, continuously logged at 1-h intervals, averaged 16.7°C (range 14.6–19.6°C) during the experiment. The temperature range was higher than that of the mesocosm, probably because this experiment was conducted during the warmer part of the season and because the creek drained the relatively warm surface water from a small (6 ha) lake.

Three upstream pools within the experimental reach served as the control. Twenty pink salmon carcasses were tethered in each of the three downstream pools, providing a total wet mass of 35, 38, and 39 kg·pool^{-1}, respectively (1:1 gender ratio). The resultant cumulative carcass concentrations and densities are given in Table 1.

Biofilm was sampled from all tiles on 3 September using the whole tile and AFDM determined as described earlier. Because the natural stream study was intended as a less rigorous counterpart of the artificial stream experiment, we decided to measure one biofilm response and chose AFDM as a more robust indicator of the microbial community. Benthic macroinvertebrates were sampled from baskets on 4 September. Baskets were removed from the riffle with a kick net held downstream to capture escapees. The contents of the net and substrate were then washed from substrate with stream water. Although baskets were filled with the same volume of substrate, some lost or gained a small amount of substrate during the experiment. For this reason, the final volume of gravel within each basket was measured and used to make a correction to invertebrate abundance. The correction averaged 21% among baskets. The substrate was washed on a 250-µm sieve; material washed from substrate and retained on the sieve was preserved in a 5% formaldehyde solution. Samples were later washed with water through 1-mm and 250-µm sieves in the laboratory. All invertebrates retained by the 1-mm sieve and a quarter of those retained by the 250-µm sieve were processed as described earlier. Representative specimens were placed in the invertebrate collection of the Pacific Northwest Research Station, Juneau, Alaska.

Experimental design and statistical analyses

Response variables for analyses were biofilm AFDM and chlorophyll *a* and benthic macroinvertebrate abundance in the mesocosm experiment and biofilm AFDM and benthic macroinvertebrate abundance in the natural stream experiment. Macroinvertebrates were identified to the lowest reliable taxon and analyzed at two general taxonomic resolutions: total taxa and family or genus.

We used a randomized split-plot design replicating six treatments and six subtreatments across five blocks for the mesocosm experiment. Treatments (one control and five amounts of salmon carcass mass) were randomly assigned to one of six channels within a table (block). Channels were whole units that each contained six equal-sized substrate baskets (subunits) for macroinvertebrate sampling. To minimize confounding effects between basket location and time, basket selection was determined using a 6 × 6 Latin square. Experimental design and data structure were similar for AFDM and chlorophyll *a* responses. All data were natural logarithm transformed to meet ANOVA assumptions and were analyzed with PROC GLM (SAS Institute Inc. 1989) at $\alpha = 0.05$. Hypothesis testing included an F test for carcass effect (control versus average of all carcass treatments) and regression analyses (slope $\neq 0$) to test for significant responses to incremental carcass mass increases. Time effect and its interaction with carcass treatments were also tested. Data (AFDM and macroinvertebrate abundance) from the natural stream were analyzed similarly to mesocosm data: F tests for carcass effect and regression analyses testing for responses to carcass mass increases.

In the case of the natural stream experiment, we caution that comparing stream reaches (with multiple samples taken from each reach) within one stream is pseudoreplication and does not provide a means of separating treatment effects from other variables. Even if significant differences are detected

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Table 2. Macroinvertebrate taxa and their corresponding functional feeding group and mean natural logarithm transformed abundance from the mesocosm and natural stream experiments and subsequent statistical output: treatment means and ANOVA and regression results.

Taxon	Functional feeding group[a]	% relative abundance[b]	Treatment mean[c]		ANOVA[c]		Linear regression test for slope ≠ 0, p
			Carcass	Control	MS_{error}	p	
Mesocosm							
Arthropoda							
Insecta							
Ephemeroptera							
Leptophlebiidae							
Paraleptophlebia	c-g	0.9	nt	nt			
Heptageniidae							
Cinygmula	c-g, sc	2.3	1.30	1.62	0.37	0.012	<0.001
Baetidae							
Baetis[d]	c-g, sc	7.4	1.87	1.14	0.48	<0.001	0.008
Ameletidae							
Ameletus	c-g, sc	0.2	nt	nt			
Plecoptera[e]		0.4	nt	nt			
Nemouridae							
Zapada[f]	sh	11.2	2.97	2.18	0.36	<0.001	0.360
Capniidae	sh	4.6	1.79	1.65	0.27	0.123	0.068
Leuctridae	sh	0.8	nt	nt			
Chloroperlidae							
Sweltsa	p	0.5	nt	nt			
Trichoptera[g]	Several	0.5	nt	nt			
Limnephilidae							
Onocosmoecus	sh	0.4	nt	nt			
Diptera[h]	Several	0.5	nt	nt			
Chironomidae	Several	69.3	4.73	3.32	0.25	<0.001	<0.001
Arachnida							
Acari	om	0.1	nt	nt			
Copepoda		0.4	nt	nt			
Annelida							
Oligochaeta	c-g	0.5	nt	nt			
All taxa		100.0	5.15	4.13	0.23	<0.001	0.091
Natural stream							
Arthropoda							
Insecta							
Ephemeroptera							
Leptophlebiidae							
Paraleptophlebia	c-g	14.2	5.52	5.05	0.12	0.009	0.519
Heptageniidae[i]		0.2	nt	nt			
Cinygmula	c-g, sc	0.9	nt	nt			
Ironodes	c-g, sc	0.6	nt	nt			
Baetidae[j]	c-g, sc	7.3	5.31	3.29	0.29	<0.001	0.159
Plecoptera							
Leuctridae	sh	0.1	nt	nt			
Nemouridae							
Zapada[k]	sh	4.3	4.44	3.94	0.10	0.003	0.514
Chloroperlidae							
Sweltsa	p	10.2	5.02	5.00	0.08	0.871	0.079
Trichoptera[l]	Several	0.5	nt	nt			
Rhyacophilidae							
Rhyacophila	p	1.1	nt	nt			
Hydropsychidae							
Hydropsyche	c-f	0.5	nt	nt			
Diptera							
Tipulidae							
Dicranota	p	0.1	nt	nt			
Ceratopogonidae	p, c-g	1.5	nt	nt			

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Table 2 (*concluded*).

Taxon	Functional feeding group[a]	% relative abundance[b]	Treatment mean[c]		ANOVA[c]		Linear regression test for slope ≠ 0, p
			Carcass	Control	MS_{error}	p	
Chironomidae	Several	53.4	7.24	5.91	0.05	<0.001	<0.001
Simuliidae							
Simulium	c-f	1.6	nt	nt			
Arachnida							
Acari	om	0.1	nt	nt			
Crustacea[m]		0.1	nt	nt			
Annelida							
Oligochaeta	c-g	1.8	nt	nt			
All taxa		100.0	7.79	6.76	0.04	<0.001	0.002

[a]c-g, collector–gatherer; c-f, collector–filterer; sc, scraper; sh, shredder; p, predator; om, omnivore (based on Merritt and Cummins 1996).
[b]Relative abundance as a percentage of all macroinvertebrates pooled across both treatments.
[c]Mesocosm, n = 5, df = 20; natural stream, n = 3, df = 16; nt, not tested (when <2% of the total abundance).
[d]*B. bicaudatus*, *B. tricaudatus*.
[e]Too small to identify.
[f]*Z. cinctepes*, *Z. columbiana*.
[g]*Micrasema*, *Ecclisomyia*, *Rhyacophila*.
[h]Simuliidae, Certatopogonidae, Tipulidae.
[i]Too small to identify.
[j]*Baetis tricaudatus*, *Diphetor hageni*.
[k]*Z. cinctipes*, *Z. oregonensis*.
[l]*Lepidostoma*, *Wormaldia*, *Polycentropus*, Brachycentridae.
[m]Amphipoda, Ostracoda.

between treated and untreated reaches, pseudoreplication does not provide grounds for making conclusive statements about treatments. Nonetheless, this approach allowed us to compare patterns detected with biota in the natural stream with those in the statistically rigorous mesocosm experiment.

Results and discussion

Mesocosm experiment

Biofilm standing stock and benthic macroinvertebrate density were significantly higher in channels that contained salmon tissue (Fig. 2). Biofilm AFDM and chlorophyll *a* ranged up to two and eight times higher, respectively, than the control treatment ($p < 0.001$); differences were more pronounced with chlorophyll *a* than with AFDM, particularly during the intermediate sampling periods (Figs. 2A and 2B). Benthic macroinvertebrate densities ranged up to five times higher in the carcass treatments versus the control (Fig. 2C; Table 2). For clarity on data structure and analyses, ANOVA output for one response variable (mean logarithm-transformed macroinvertebrate abundance) is presented in Table 3.

There was also a significant time effect on AFDM, chlorophyll *a*, and benthic macroinvertebrates ($p < 0.001$) (Fig. 2). This in part appeared to be related to treatments but also to a sudden sediment pulse that passed through the channels during a spate 30 days into the experiment. Following the spate, we gently agitated by hand the surface substrate to facilitate flushing out some of the fine sediment that settled in the channels. We believe that the sediment and agitation contributed to the decline of AFDM (loosely attached) and invertebrate levels at about day 30 (Figs. 2A and 2C); however, their levels soon rebounded. Chlorophyll *a* did not appear to be negatively affected by the sediment pulse (Fig. 2B), and neither did the carcass loadings (i.e., treatments).

As was the case in a previous study (Wipfli et al. 1998), sloughing was apparent and grazing invertebrates were seen on clay tiles apparently removing biofilm as it developed, which probably reduced biofilm standing stock throughout the experiment. There appeared to be an emerging pattern of higher benthic macroinvertebrate density through time as a function of carcass loading (most apparent at the end of the study): higher densities at higher loading (Fig. 2C). If this pattern was real, it suggests that more nutrients may support more invertebrates in the longer term (>1.5 months), at least at these tested loading rates. Unfortunately, scavenging of salmon by bears forced us to start the experiment later than we intended and we therefore could not explore the loading effect beyond 60 days.

While there were large increases in AFDM, chlorophyll *a*, and macroinvertebrate abundance with carcass treatment at the lowest loading (1.45 kg), less dramatic responses were seen at subsequent higher loading levels (Fig. 3). The regression slope of 0.42 for chlorophyll *a* ($p = 0.015$) was significant across the 1.45–7.25 kg carcass loading; slopes were not significant for AFDM and benthic macroinvertebrate abundance ($p > 0.05$). These data suggest that a trophic capacity was generally reached somewhere between 0.00 and 1.45 kg carcass mass. These loading levels represent a common range of combined salmon spawner densities in Southeast Alaska and encompass the upper range of loading observed in many medium-sized rivers close to salt water where pink and chum salmon (*Oncorhynchus keta*) generally spawn at their highest densities (Groot and Margolis 1991). However, these data represent a two-month time frame. Although highly speculative, longer term effects (>two months) could be different depending on the system's storage capacity (Bilby et al. 1996; Kline et al. 1997). Some nutrients may be stored initially and released through time. In other words,

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Table 3. ANOVA results for carcass and time effect and carcass treatment × time interaction from benthic macroinvertebrate mean natural logarithm transformed abundance data from the mesocosm experiment.

	df	Type III SS	MS	*F*	*p*
Table	4	2.48	0.62		
Treatment	5	27.36	5.47	24.26	<0.001
Orthogonal contrasts					
Carcass treatments versus control	1	26.92	26.92	119.37	<0.001
Regression slope ≠ 0	1	0.71	0.71	3.15	0.094
Lack of fit	3	0.41	0.14	0.61	0.618
Error (table × treatment)	20	4.51	0.23		
Time	5	19.95	3.99	21.36	<0.001
Treatment × time	25	7.05	0.28	1.51	0.071
Error (table × treatment × time)	116	21.67	0.19		
Total	175	83.02			

Note: Orthogonal contrast SS do not add up to treatment SS due to some missing data from one channel.

higher carcass loads may appear "excessive" beyond what the biota can immediately utilize in a given system in the short term (e.g., in autumn); however, the higher loads may provide nutrients that are stored (Bilby et al. 1996) and then released over time and utilized by biota over the longer term. This warrants further study.

Within the benthic macroinvertebrate community, taxa responded differently to carcass loading (Fig. 4). Three of the five common taxa (each comprising >2% of the total abundance) responded positively to carcass addition (control versus all loading treatments): Chironomidae (comprising a mixture of functional feeding groups), *Zapada* (shredders), and *Baetis* (collector–gatherers and scrapers) (Table 2). But over the five carcass loading rates, these three taxa responded differently. Chironomidae densities continued to increase over increased loading ($p < 0.001$), *Baetis* significantly decreased ($p = 0.008$), and *Zapada* showed no change to loading ($p = 0.36$). In addition, the shredder Capniidae did not respond to treatments ($p > 0.05$), while *Cinygmula* significantly decreased with increased loading ($p < 0.001$). These taxon-specific responses may have been due to food quality changes in the biofilm layer; we did observe a color succession from green to brown and gray of the biofilm across treatments throughout the experiment. Differences could also have been a reflection of food acquisition differences among instars or life history differences among taxa.

Natural stream experiment

Similar patterns were observed with biota in the natural stream as recorded in the mesocosm (Fig. 5). Both biofilm AFDM and benthic macroinvertebrate densities significantly increased following carcass addition; AFDM was about five times greater at the highest loading level versus control reaches ($p < 0.001$) and benthic macroinvertebrate densities about three times greater ($p < 0.001$). Densities of the five most common benthic macroinvertebrate taxa increased significantly ($p < 0.001$ to $p = 0.009$) with carcass addition (Fig. 6), except *Sweltsa*, a predatory stonefly, which showed no change ($p = 0.87$). Further, the combined benthic macroinvertebrate community level increase with carcass loading was statistically significant ($p = 0.002$). Chironomidae were responsible for the observed benthic macroinvertebrate community level response (Fig. 6); they were the most abundant taxa (Table 2) and were the only taxa that showed a significant response to loading ($p < 0.001$). Even though the highest loading rate was a low carcass density (2 spawners·m^{-2}) relative to the natural range of spawner densities in the vicinity (0–20 spawners·m^{-2}) (Wipfli et al. 1998; S. Heinl, Alaska Department of Fish and Game, personal communication), it had a large effect on both trophic categories, biofilm and invertebrates. AFDM and benthic macroinvertebrate densities were still increasing at the highest carcass load ($p = 0.024$ and $p = 0.002$, respectively) and not reaching a trophic capacity as seen in the mesocosm. We believe that this was a function of the low loading rates in the natural stream versus the wide load range tested in the artificial streams (Table 1).

Ecological considerations and management implications

These results illustrate important concepts, MDN subsidy of stream food webs and trophic capacities, with implications for fisheries and ecosystem management and demonstrate the need for a better understanding of the ecological role of MDN in freshwater and riparian systems. This and other studies suggest that spawners may elevate stream productivity through stimulating lower trophic levels that support juvenile salmon and other fishes (Bilby et al. 1996; Wipfli et al. 1998). More spawners translate into higher nutrient loads and more energy-rich detritus (Mathisen et al. 1988) for scavengers and detritivores, in turn potentially providing more prey for fish. The large and rapid increase in biofilm and invertebrate abundance in this study shows that additional nutrients supported more benthos. In a parallel study, growth rates of *Psychoglypha* (Trichoptera: Limnephilidae) larvae were higher in carcass-enriched habitats (D.T. Chaloner, unpublished data), also reflecting increased productivity. More freshwater prey should support faster growing and more and larger juvenile salmonids (Groot and Margolis 1991), in turn reducing their time to smoltification and increasing overwinter survival. Any combination of these processes should elevate smolt production. Trophic capacities, as demonstrated in this study and present at some level in all natural systems, should ultimately control the amount of prey that a given system can produce. In these nutrient-limited systems of the Pacific Northwest, British Columbia, and Alaska (Gregory et al. 1987; Kyle et al.

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Fig. 3. Mean (A) AFDM, (B) chlorophyll *a* density, and (C) benthic macroinvertebrate density in the mesocosm experiment averaged over time across six salmon carcass loading levels. Error bars represent ±1 SEM.

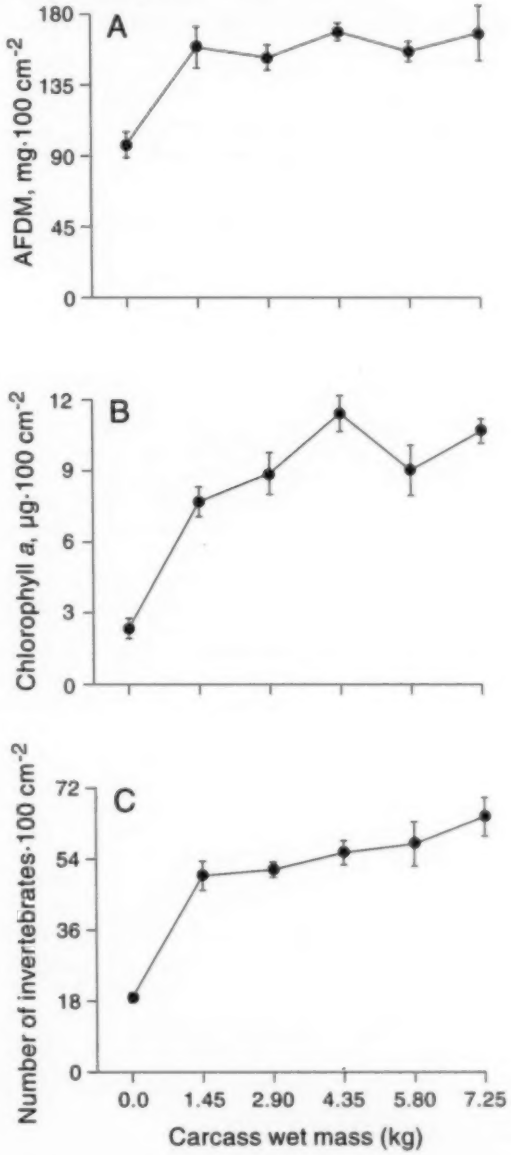
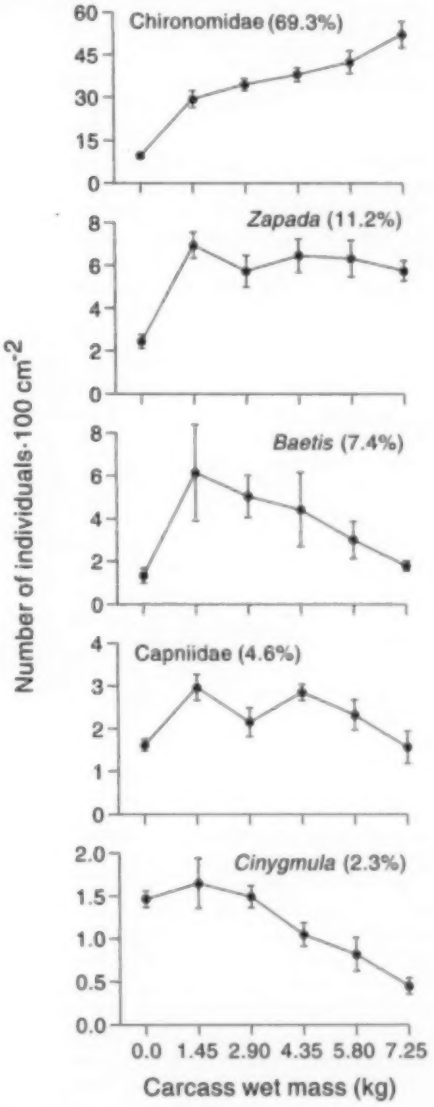


Fig. 4. Mean densities of the most common (by percentage) benthic macroinvertebrates in the mesocosm experiment averaged over time across six salmon carcass loading levels. Error bars represent ±1 SEM.



1997), even small nutrient pulses can be expected to have a large effect on food web productivity.

The amount of nutrients and their ratio necessary to sustain productivity may depend on preexisting physical, chemical, and biological system-specific conditions and their interactions (Fig. 7). The effects of nutrient loading on productivity, whether it be naturally by anadromous fishes or artificially by placing spawned-out hatchery carcasses (Levy 1997) or artificial nutrient additions (Ashley and Slaney 1997; Kyle et al. 1997), will likely depend on existing geology-based nutrient concentrations (Coleman and Dahm 1990), light levels and water temperature (Allan 1995), nutrient storage capacity (Bilby et al. 1996), among several other processes, specific to a given reach, subbasin, or watershed. Productivity of a system that is not nutrient limited will probably respond less to nutrient loading, as observed by Rand et al. (1992) in Lake Ontario tributaries. Nutrient–light interactions (Borchardt 1996) will also likely affect productivity, especially in small- to medium-sized forested streams (Vannote et al. 1980) common throughout the Pacific Northwest. More light for photosynthesis at a given nutrient level should increase primary production and, in

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Fig. 5. Mean (A) AFDM and (B) benthic macroinvertebrate densities in riffle habitats in the natural stream, contrasting upstream control (○) and downstream carcass-enriched (●) reaches. Error bars represent ±1 SEM.

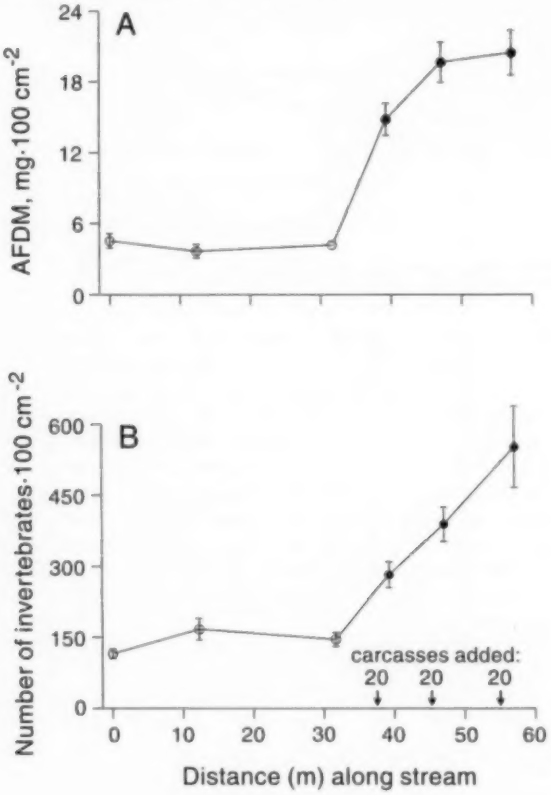
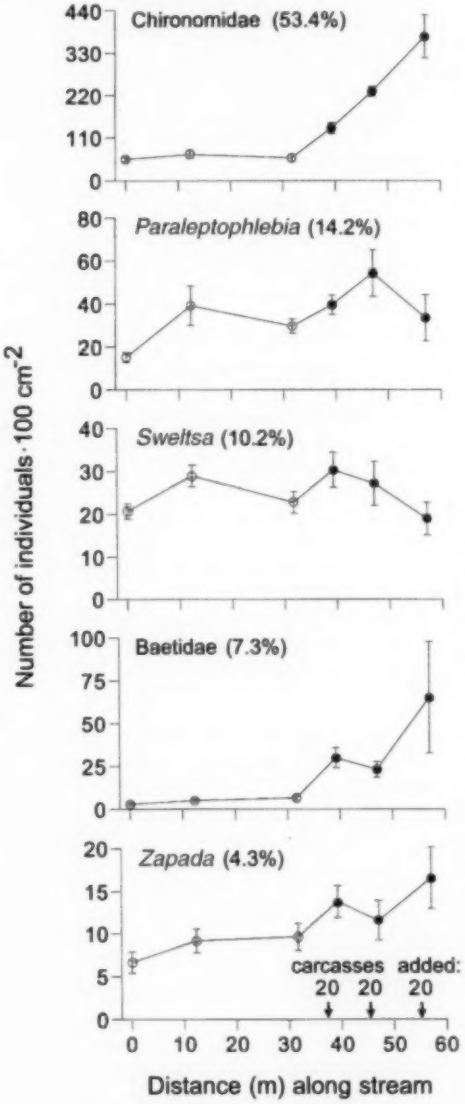


Fig. 6. Mean densities of the most common (by percentage) benthic macroinvertebrates in the natural stream, contrasting upstream control (○) and downstream carcass-enriched (●) reaches. Error bars represent ±1 SEM.



turn, grazer abundance. Additionally, higher water temperature, to a limit, may also increase metabolic and developmental rates of biofilm and invertebrates (Allan 1995), ultimately affecting their production.

Redistribution of salmon from streams onto land through flooding and vertebrate scavenging (Cederholm et al. 1989; Ben-David et al. 1998) may influence community responses to spawner loading. Nutrients that would otherwise leach directly into streams are often instead spread throughout the terrestrial environment via whole or fragmented carcasses or carnivore feces at various distances from the stream (Cederholm et al. 1989; Ben-David et al. 1998; Willson et al. 1998). In instances where carnivores remove all or nearly all of the spawners from a stream (M.S. Wipfli, personal observation), the stream biota may receive little nutrients directly. However, the nutrients should reach the stream system over time as they leach through the soil and hyporheos or after they are taken up by streamside vegetation and then enter the stream as plant litter (Vannote et al. 1980) and terrestrial invertebrates (Wipfli 1997).

Amplitude (salmon abundance), duration, and timing of the salmon runs and the salmon species present may also influence the ecological response to loading within watersheds. More salmon mean more nutrients. Runs spanning more time (e.g., six versus two months) will meter out nutrient additions over a greater portion of the year. Additionally, earlier runs will have an earlier effect on biota, potentially providing more prey for fishes through the growing season (before winter). Later runs (e.g., fall and winter) may have less immediate effects on stream biota because of prevailing low light levels (i.e., decreased photosynthesis of periphyton), colder water (i.e., reduced growth and developmental rates of microbes and macroinvertebrates and reduced feeding by fishes), or freezing. Thus, effects of late runs may be felt more during the following growing season pro-

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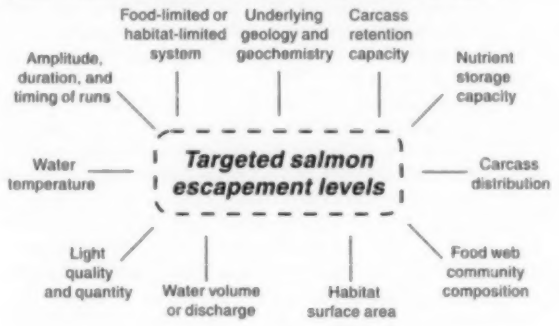
Fig. 7. Conceptual model showing major system-specific physical, chemical, and biological features that may influence aquatic community responses to MDN from salmon runs.



vided some nutrients are stored through winter. Also, species-dependent spatial effects could be prominent. Coho (*Oncorhynchus kisutch*), chinook (*Oncorhynchus tshawytscha*), and sockeye salmon (*Oncorhynchus nerka*) generally spawn farther up watersheds than do pink and chum salmon (Groot and Margolis 1991); systems receiving mostly the latter will receive fewer nutrients farther into the watershed than systems receiving coho, chinook, or sockeye salmon. The species mix of spawners within a system could also play a role. A system receiving one or few species of salmon will likely get a much shorter nutrient pulse than one supporting early (sockeye and chinook salmon), midseason (chum and pink salmon), and late (coho salmon) spawners (Groot and Margolis 1991). Further, physical disturbance of the streambed by spawners during redd construction (Montgomery et al. 1996) may locally reduce biofilm and invertebrate densities. However, because redd construction is usually limited to certain areas within the stream and watershed, depending on spawner species and their densities, effects will be spatially limited. In addition, because spawning activities are limited to only a fraction of the year, with peak spawning activities generally occurring over several weeks, temporal effects of redd construction on benthos should be short-lived. The bulk of the nutrients probably leach into stream water, becoming available to benthos or going into storage, after redd construction when most of the salmon are dead or dying.

Physical characteristics of streams could also influence nutrient effects. High versus low discharges will dramatically affect carcass and nutrient concentrations in stream water (see Table 1, Natural stream). Stream gradient will likely affect carcass and nutrient retention; low-gradient systems may have a smaller flushing effect than high-gradient ones. Cederholm and Peterson (1985) and Cederholm et al. (1989) found that woody debris in streams functions to retain carcasses; more debris means more carcass and nutrient retention and may be particularly important during high flows. Boulders, pools, side channels, and lakes may also be important carcass storage areas. Carcasses buried in the hyporheic zone from spates (frequent during that time of year in Southeast Alaska) may also serve as longer term nutrient sources. Shallow hyporheic layers will have less retentive capacity than deep ones. The amount of wetted streambed and hyporheic area available for biofilm and macroinvertebrate colonization is also a factor that may influence nutrient effects in streams. More habitat surface area (e.g., streambed, woody debris, and interstitial spaces) will provide more space for colonists. Systems with extensive hyporheic zones extending well into the riparian flood plain could have large storage capacities. Additionally, fine-textured sediments contain more surface area for physical sorption (Bilby et al. 1996) than coarse-textured substrates, a function of the surface area to volume ratio.

A key biological factor is food web community structure, a function of stream size and energy pathways (Vannote et al. 1980). Grazers will utilize the microbial layer that develops following nutrient influx, as seen in this study. Sloughed microbial cells and organic particles will be available to collector–gatherers and collector–filterers. Predatory macroinvertebrates should respond to increased prey abundance, depending on their prey preference and on which taxa respond the most to nutrients and organic inputs. Wallace et al. (1997) documented negative effects of removing a large organic food base (allochthonous leaf litter) on multiple trophic levels in a forest stream, with effects felt from detritivores up through predators. Shredders, characteristic of small forested streams (Vannote et al. 1980), may respond the least if they are not utilizing the biofilm layered on their organic food base (e.g., leaves). On the other hand, they may respond dramatically if they consume energy-rich salmon tissue, particularly if they are food limited. Bilby et al. (1996) found 0% marine carbon uptake by shredders in two anadromous streams in Washington. River continuum theory (Vannote et al. 1980) predicts abundant shredders and few grazers in canopied streams, common habitats for spawning salmon in Southeast Alaska, and the reverse in larger streams. Communities in small forested streams dominated by shredders should respond less to MDN than grazer-dominated communities unless the species possess omnivorous habits.

Clearly, we need a better understanding of the role that MDN from salmon and other anadromous fishes play in freshwater and terrestrial ecosystems, including energy flow, nutrition, food web interactions and complexity, productivity, and biodiversity. Watersheds likely all respond to marine nutrient loading but probably do so differently; system-specific chemical, physical, and biological processes operating within a watershed will likely interact to regulate nutrient loading effects. More salmon may mean more nutrients but not necessarily greater availability; high loading may saturate the ability of a system to sequester and efficiently use nutrients, as demonstrated in this study. A given escapement level, e.g., 100 000 spawners, may not saturate the productive capacity of a system that has a large storage capacity (i.e., large hyporheic zone), that is autotrophically driven (i.e., sparse riparian canopy that provides much sunlight penetration and warmer water), and that is strongly nutrient limited. On the other hand, the productive capacity of a system characterized by bedrock (i.e., minimal storage capacity) and dense riparian canopy, and is not nutrient limited, will probably saturate more quickly at the same escapement level or spawner density. Depending on their preexisting character (Fig. 7), systems may need to be managed differ-

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ently at various scales: subbasin, watershed, or regionally (based on ecology or underlying geology). Nonetheless, a more holistic fisheries and ecosystem management approach requires better information on the dependence on and consequences of marine-derived subsidies in freshwater and terrestrial ecosystems.

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References

Allan, J.D. 1995. Stream ecology: structure and function of running waters. Chapman and Hall, New York.

Ashley, K.I., and Slaney, P.A. 1997. Accelerating recovery of stream, river and pond productivity by low-level nutrient enrichment. *In* Fish habitat rehabilitation procedures. *Edited by* P.A. Slaney and D. Zaldokas. Watershed Restoration Tech. Circ. No. 9, B.C. Ministry of Environment, Lands and Parks and Ministry of Forests, Vancouver, B.C.

Baker, T.T., Wertheimer, A.C., Burkett, R.D., Dunlap, R., Eggers, D.M., Fritts, E.I., Gharrett, A.J., Holmes, R.A., and Wilmot, R.L. 1996. Status of Pacific salmon and steelhead escapements in southeastern Alaska. Fisheries (Bethesda), **21**: 6–18.

Ben-David, M., Hanley, T.A., Klein, D.R., and Schell, D.M. 1998. Fertilization of terrestrial vegetation by spawning Pacific salmon: the role of flooding and predator activity. Oikos, **83**: 47–55.

Bilby, R.E., Fransen, B.R., and Bisson, P.A. 1996. Incorporation of nitrogen and carbon from spawning coho salmon into the trophic system of small streams: evidence from stable isotopes. Can. J. Fish. Aquat. Sci. **53**: 164–173.

Bilby, R.E., Fransen, B.R., Bisson, P.A., and Walter, J.K. 1998. Response of juvenile coho salmon (*Oncorhynchus kisutch*) and steelhead (*Oncorhynchus mykiss*) to the addition of salmon carcasses to two streams in southwestern Washington, U.S.A. Can. J. Fish. Aquat. Sci. **55**: 1909–1918.

Borchardt, M.A. 1996. Nutrients. *In* Algal ecology. *Edited by* R.J. Stevenson, M.L. Bothwell, and R.L. Lowe. Academic Press, San Diego, Calif. pp. 183–227.

Cederholm, C.J., and Peterson, N.P. 1985. The retention of coho salmon (*Oncorhynchus kisutch*) carcasses by organic debris in small streams. Can. J. Fish. Aquat. Sci. **42**: 1222–1225.

Cederholm, C.J., Houston, D.B., Cole, D.L., and Scarlett, W.J. 1989. Fate of coho salmon (*Oncorhynchus kisutch*) carcasses in spawning streams. Can. J. Fish. Aquat. Sci. **46**: 1347–1355.

Coleman, R.L., and Dahm, C.N. 1990. Stream geomorphology: effects on periphyton standing crop and periphyton. J. North Am. Benthol. Soc. **9**: 293–302.

Cummins, K.W., Sedell, J.R., Swanson, F.J., Minshall, G.W., Fisher, S.G., Cushing, C.E., Peterson, R.C., and Vannote, R.L. 1983. Organic matter budgets for stream ecosystems. *In* Stream ecology: application and testing of general ecological theory. *Edited by* J.R. Barnes and G.W. Minshall. Plenum, New York. pp. 299–353.

Gregory, S.V., and Bisson, P.A. 1997. Degradation and loss of anadromous salmonid habitat in the Pacific Northwest. *In* Pacific salmon and their ecosystems: status and future options. *Edited by* D.J. Stouder, P.A. Bisson, and R.J. Naiman. Chapman and Hall, New York. pp. 277–314.

Gregory, S.V., Lamberti, G.A., Erman, D.C., Koski., K.V., Murphy, M.L., and Sedell, J.R. 1987. Influence of forest practices on aquatic production. *In* Streamside management: forestry and fishery interactions. *Edited by* E.O. Salo and T.W. Cundy. Institute of Forest Resources, University of Washington, Seattle, Wash.

Groot, C., and Margolis, L. (*Editors*). 1991. Pacific salmon life histories. University of British Columbia Press, Vancouver, B.C.

Halupka, K.C., Bryant, M.D., Willson, M.F., and Everest, F.H. 1999. Biological characteristics and population status of anadromous salmon in Southeast Alaska. Gen. Tech. Rep. PNW-GTR-468. U.S. Dep. Agriculture, Forest Service, Pacific Northwest Research Station, Portland, Oreg. In press.

Hesson, D.O. 1997. Stoichiometry in food webs — Lotka revisited. Oikos, **79**: 195–200.

Kline, T.C., Goering, J.J., Mathisen, Q.A., and Poe, P.H. 1990. Recycling of elements transported upstream by runs of Pacific salmon: I. $\delta^{15}N$ and $\delta^{13}C$ evidence in Sashin Creek, southeastern Alaska. Can. J. Fish. Aquat. Sci. **47**: 136–144.

Kline, T.C., Jr., Goering, J.J., and Piorkowski, R.J. 1997. The effect of salmon carcasses on Alaskan freshwaters. *In* Freshwaters of Alaska: ecological syntheses. *Edited by* A.M. Milner and M.W. Oswood. Springer-Verlag, New York. pp. 179–204.

Kyle, G.B., Koenings, J.P., and Edmundson, J.A. 1997. An overview of Alaska lake-rearing salmon enhancement strategy: nutrient enrichment and juvenile stocking. *In* Freshwaters of Alaska: ecological syntheses. *Edited by* A.M. Milner and M.W. Oswood. Springer-Verlag, New York. pp. 205–227.

Levy, S. 1997. Pacific salmon bring it all back home. BioScience, **47**: 657–660.

Maser, C., and Sedell, J.R. 1994. From the forest to the sea: the ecology of wood in streams, rivers, estuaries, and oceans. St. Lucie Press, Delray Beach, Fla.

Mathisen, O.A., Parker, P.L., Goering, J.J., Kline, T.C., Poe, P.H., and Scalan, R.S. 1988. Recycling of marine elements transported into freshwater systems by anadromous salmon. Verh. Int. Ver. Limnol. **23**: 2249–2258.

Merritt, R.W., and Cummins, K.W. (*Editors*). 1996. An introduction to the aquatic insects of North America. Kendall/Hunt, Dubuque, Iowa.

Montgomery, D.R., Peterson, N.P., Schuett-Hames, D., and Quinn, T.P. 1996. Stream-bed scour, egg burial depths, and the influence of salmonid spawning on bed surface mobility and embryo survival. Can. J. Fish. Aquat. Sci. **53**: 1061–1070.

Parmenter, R.R., and Lamarra, V.A. 1991. Nutrient cycling in a freshwater marsh: the decomposition of fish and waterfowl carrion. Limnol. Oceanogr. **36**: 976–987.

Polis, G.A., Anderson, W.B., and Holt, R.D. 1997. Toward an integration of landscape and food web ecology: the dynamics of spatially subsidized food webs. Annu. Rev. Ecol. Syst. **28**: 289–316.

Quinn, T.P., and Peterson, N.P. 1996. The influence of habitat complexity and fish size on over-winter survival and growth of individually marked juvenile coho salmon (*Oncorhynchus kisutch*) in Big Beef Creek, Washington. Can. J. Fish. Aquat. Sci. **53**: 1555–1564.

Rand, P.S., Hall, C.A.S., McDowell, W.H., Ringler, N.H., and Kennon. J.G. 1992. Factors limiting primary productivity in Lake

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Ontario tributaries receiving salmon migrations. Can. J. Fish. Aquat. Sci. **49**: 2377–2385.

SAS Institute Inc. 1989. SAS/STAT user's guide, version 6, edition 4. Vol. 2. SAS Institute Inc., Cary, N.C.

Schuldt, J.A., and Hershey, A.E. 1995. Effect of salmon carcass decomposition on Lake Superior tributary streams. J. North Am. Benthol. Soc. **14**: 259–268.

Slaney, T.L., Hyatt, K.D., Northcote, T.G., and Fielden, R.J. 1996. Status of anadromous salmon and trout in British Columbia and Yukon. Fisheries (Bethesda), **21**: 20–35.

Steinman, A.D., and Lamberti, G.A. 1996. Biomass and pigments of benthic algae. *In* Methods in stream ecology. *Edited by* F.R. Hauer and G.A. Lamberti. Academic Press, San Diego, Calif. pp. 295–313.

Vannote, R.L., Minshall, G.W., Cummins, K.W., Sedell, J.R., and Cushing, C.E. 1980. The river continuum concept. Can. J. Fish. Aquat. Sci. **37**: 130–137.

Wallace, J.B., Eggert, S.L., Meyer, J.L., and Webster, J.L. 1997. Multiple trophic levels of a forest stream linked to terrestrial litter inputs. Science (Washington, D.C.), **277**: 102–104.

Wertheimer, A.C. 1997. Status of Alaska salmon. *In* Pacific salmon and their ecosystems: status and future options. *Edited by* D.J. Stouder, P.A. Bisson, and R.J. Naiman. Chapman and Hall, New York. pp. 179–197.

Willson, M.F., Gende, S.M., and Marston, B. 1998. Fishes and the forest: expanding perspectives on fish–wildlife interactions. BioScience, **48**: 455–462.

Wipfli, M.S. 1997. Terrestrial invertebrates as salmonid prey and nitrogen sources in streams: contrasting old-growth and young-growth riparian forests in southeastern Alaska, U.S.A. Can. J. Fish. Aquat. Sci. **54**: 1259–1269.

Wipfli, M.S., Hudson, J., and Caouette, J. 1998. Influence of salmon carcasses on stream productivity: response of biofilm and benthic macroinvertebrates in southeastern Alaska, U.S.A. Can. J. Fish. Aquat. Sci. **55**: 1503–1511.

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Influence of growth and survival costs of reproduction on Atlantic cod, *Gadus morhua*, population growth rate

Jeffrey A. Hutchings

Abstract: A stochastic, age-structured life history model was used to examine how age at maturity (θ), pre- (Z_{imm}) and postreproductive (Z_{mat}) mortality, and postreproductive growth rate can affect maximum reproductive rates of fish at low population size. Simulations suggest that annual (r) and per-generation (R_0) metrics of population growth for Newfoundland's northern Grand Bank Atlantic cod, *Gadus morhua*, are primarily influenced by changes to mortality prior to and following reproduction. At observed weights at age and Z_{mat} = 0.2, r ranged between 0.135 and 0.164 for cod maturing at between 4 and 7 years. Incremental increases in either Z_{imm} or Z_{mat} of 0.1 were associated with 0.03–0.05 reductions in r. To effect similar reductions, individual growth rate would have to decline by approximately one half. At observed weights at age, increases in Z_{mat} from 0.20 to 0.45 increased the probability of negative per-generation growth from 3 to 26% for cod maturing at 4 years and from 6 to 46% for cod maturing at 7 years. Thus, even in the absence of fishing mortality, little or no population growth by Atlantic cod may not be unexpected in the presence of environmental stochasticity, particularly when accompanied by increases in mortality and declining individual growth.

Résumé : Un modèle stochastique du cycle vital selon l'âge a été utilisé pour étudier de quelle manière l'âge à la maturité (θ), la mortalité avant la reproduction (Z_{imm}) et après la reproduction (Z_{mat}), et le taux de croissance après la reproduction peuvent influer sur le taux maximal de reproduction des poissons d'une population peu abondante. Selon les simulations, les mesures annuelles (r) et les mesures par génération (R_0) de la croissance de la population de la morue (*Gadus morhua*) de la partie nord du Grand Banc de Terre-Neuve sont surtout influencées par des modifications de la mortalité avant et après la reproduction. Pour les poids selon l'âge observés et une valeur de Z_{mat} = 0,2, r était compris entre 0,135 et 0,164 dans le cas de la morue qui atteint la maturité entre 4 et 7 ans. Les augmentations par échelon de 0,1 de Z_{imm} ou de Z_{mat} étaient associées à des baisses du facteur r comprises entre 0,03 et 0,05. Pour obtenir des baisses similaires, le taux de croissance individuel devrait chuter d'environ la moitié. Pour des valeurs observées de poids selon l'âge, des hausses de Z_{mat} variant de 0,20 à 0,45 augmentaient la probabilité d'une croissance par génération négative de 3 à 26 % chez la morue qui atteint la maturité à l'âge de 4 ans, et de 6 à 46 %, chez celle qui atteint la maturité à l'âge de 7 ans. En conséquence, même en l'absence de mortalité due à la pêche, une croissance faible, voire nulle, de la population de morue en présence de la stochasticité environnementale n'est pas à exclure, en particulier lorsqu'elle est accompagnée d'une augmentation de la mortalité et d'une baisse de la croissance individuelle.

[Traduit par la Rédaction]

Introduction

The parameter of greatest import in population dynamics, evolutionary ecology, and evolutionary biology is rate of increase. The fitness of a genotype, for example, is defined as the rate at which it transmits its genes to future generations relative to that of other genotypes in the same population (Stearns 1992). From an evolutionary perspective, Bell (1997) has argued that rate of self-replication is the only attribute that can be selected directly. And the ability of a population to persist in the face of demographic and environmental stochasticity in survival and fecundity appears to be best reflected by that population's rate of increase (Lande 1993). This point is of particular importance to commercially harvested populations for which spatial and temporal variation in natural mortality is compounded by spatial and temporal variation in mortality due to harvesting.

From a conservation perspective, quantifying rate of increase is perhaps most important when a population has been reduced to a small fraction of the size at which it was presumed stable. For commercially harvested fish populations, the ability of a stock to "recover," i.e., return to some usually arbitrary level of abundance after population collapse, and the time required for such recovery to occur are both functions of that stock's rate of increase at low abundance.

The most widely accepted metrics of maximum population growth rate are the instantaneous, or intrinsic, rate of natural increase, given as r, and the net reproductive rate, R_0 (Roff 1992; Stearns 1992; Charlesworth 1994). The

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units of the former parameter are "individuals per individual per unit time" (usually per year), while those of the latter are "individuals per individual per generation." Thus, r is an annual rate of increase, expressed as a percentage by $(\exp(r) - 1)100$, while R_0 is a per-generation rate of increase.

Since their collapse in the early 1990s, most of Canada's Atlantic cod, *Gadus morhua*, fisheries remain closed to commercial exploitation because of low realized rates of population increase. Among the stocks, northern cod, extending from southern Labrador to the northern half of the Grand Bank (i.e., Northwest Atlantic Fishery Organization (NAFO) Divisions 2J, 3K, and 3L), once supported Canada's largest cod fishery. Based on their analysis of the relationship between spawner biomass and recruitment of the 1962–1989 cohorts, Myers et al. (1997*a*) estimated r to be 0.26 (correction made by R.A. Myers to the 0.17 estimate provided by Myers et al. 1997*a*), corresponding to a maximum annual rate of increase for northern cod of 30%. The observation that this stock in the late 1990s appears not to have grown significantly since a commercial fishing moratorium was imposed in 1992 may reflect a lack of sensitivity of research surveys to detect low rates of increase for a widely distributed stock at low abundance. A second possibility is that Myers et al.'s (1997*a*) maximum growth estimate rate of $r = 0.26$ may have been overestimated or may no longer be representative of a population whose age structure has been severely truncated. A third possibility, and one that I wish to explore further here, is that the observed rate of change in abundance of northern cod, and potentially other Northwest Atlantic cod stocks, since 1992 may not be unexpected given natural, stochastic variation in the life history variables that directly influence the stock's rate of increase.

I use an age-structured life history model to estimate rates of population increase and their associated frequency distributions for a population of Atlantic cod in the Northwest Atlantic. The stochastic version of this model incorporates observed or estimated variation in the following: (*i*) age-specific increases in weight, (*ii*) survival from birth to age 3 years, (*iii*) annual prereproductive survival probabilities, (*iv*) annual survival costs of reproduction, and (*v*) annual growth costs of reproduction. The costs of reproduction represented by the fourth and fifth parameters reflect the observation that, for indeterminately growing organisms, reproduction at age x usually results in reduced survival to, and slower rates of growth at, subsequent ages (Roff 1992; Stearns 1992; Hutchings 1993).

Based on fecundity and growth data available for cod inhabiting the northern half of Newfoundland's Grand Bank, the region containing most of the research survey biomass for northern cod in the 1990s (Lilly et al. 1998), my objectives were to (*i*) use a stochastic, age-structured life history model to estimate maximum population growth rates (r and R_0) for Atlantic cod, (*ii*) quantify the effects of age at maturity, pre- and post-reproductive mortality, and growth costs of reproduction on population growth rate, and (*iii*) evaluate how per-generation probabilities of population doubling, i.e., $\Pr(R_0 > 2)$, and of population decline, i.e., $\Pr(R_0 < 1)$, vary with reproductive costs. This use of a stochastic model builds upon work pioneered by Lewontin and Cohen (1969), and extended by Roff (1974), who modelled the effects of random fluctuations in population growth rate on expectations of population size and probabilities of extinction.

Materials and methods

Age-specific rates of survival and fecundity can be used to estimate rates of population increase in an age-structured population. These data are expressed as l_x, the probability of surviving from birth until the beginning of the breeding season at age x, and m_x, the number of eggs produced by a female breeding at age x. The intrinsic rate of population increase is calculated from the discrete-time version of the Euler–Lotka equation:

$$(1) \qquad 1 = \sum_{x=\theta}^{x=\tau} l_x m_x e^{-rx}$$

where θ represents age at maturity and τ represents age at death ($\tau = 21$ years in the present analysis). The net reproductive rate, R_0, is given by

$$(2) \qquad R_0 = \sum_{x=\theta}^{x=\tau} l_x m_x.$$

Probability distributions of the intrinsic rate of increase cannot be described by a stochastic model of population growth because of the analytical constraint that r can only be calculated by iteration. However, the intrinsic rate of natural increase can be approximated by the natural logarithm of the net reproductive rate discounted by generation time, G, as

$$(3) \qquad r = \ln(R_0)/G$$

where

$$(4) \qquad G = \left(\sum l_x m_x x\right) \Big/ \left(\sum l_x m_x\right)$$

Probability distributions for all estimates of R_0 presented here were generated from 2000 randomizations. The highly positive skew of these distributions necessitated the calculation of r from median estimates of R_0.

Age-specific fecundity

Fecundity was calculated from the relationship between gilled-and-gutted weight and number of eggs per female reported by May (1967) for Atlantic cod collected from several locations off northeastern Newfoundland, including the Grand Bank. Gilled-and-gutted weight, w_{gg}, was estimated from round weight, w, using a relationship derived from samples of prespawning female cod captured between March and May in NAFO Division 3L between 1978 and 1989, i.e., $w = 1.45w_{gg} - 0.37$ (G.R. Lilly, Department of Fisheries and Oceans, P.O. Box 5667, St. John's, NF A1C 5X1, Canada, personal communication). Substituting this equation into May's (1967) relationship, age-specific fecundities, m_x, were calculated as

$$(5) \qquad m_x = (0.48((w_x + 0.37)/1.45) + 0.12) \times 10^6.$$

Age-specific growth rate

Individual growth rates were approximated by expected changes to size at age throughout an individual's lifetime. The resultant weights at each age x were calculated by initially multiplying the average weight at age 4 years by the expected age-specific increase in body size between ages 4 and 5 years, i.e., w_5/w_4, to obtain an estimate of weight at age 5 years, then multiplying this estimate of weight at age 5 years by w_6/w_5 to obtain an estimate of weight at age 6 years, and so on until the age of 20 years. Empirical esti-

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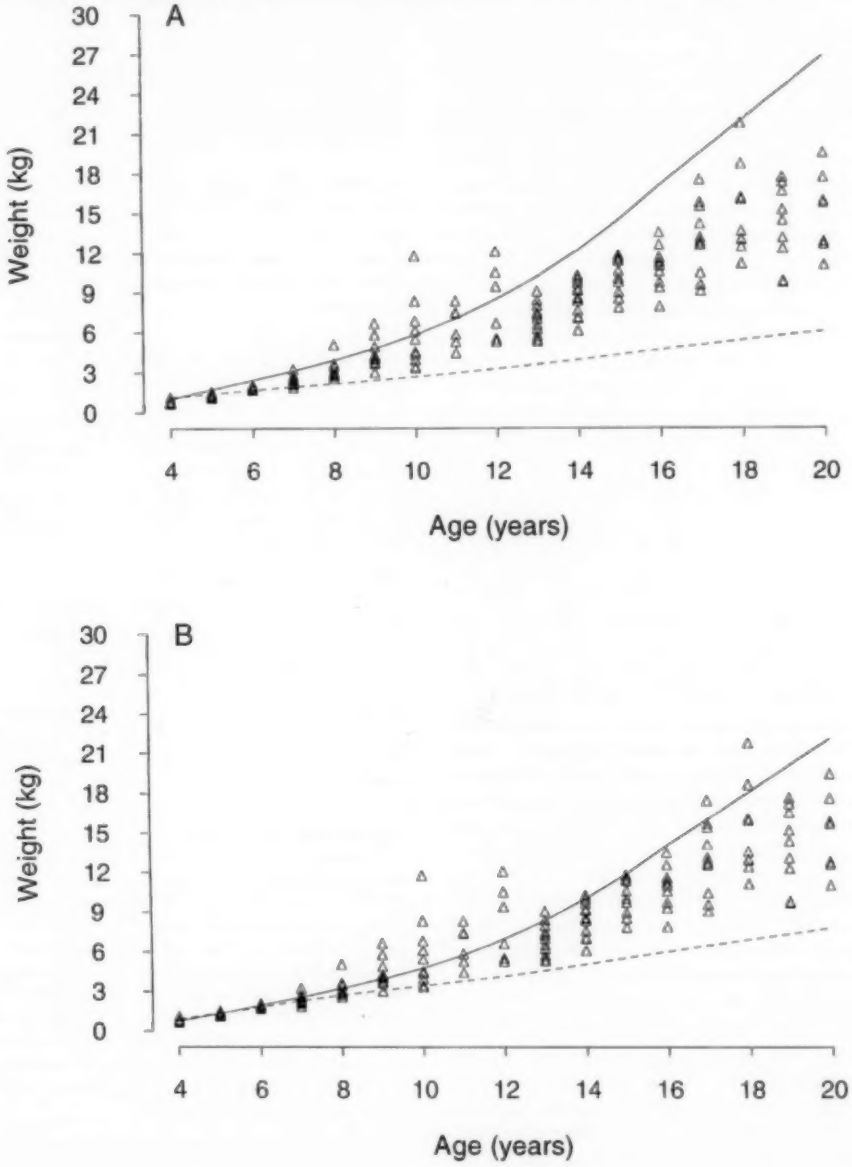
Fig. 1. Observed and modelled weights at age for Atlantic cod maturing at (A) 4 years and (B) 7 years. Triangles represent weights measured for 4- to 12-year-old cod captured on northern Grand Bank (NAFO Division 3L) by research surveys between 1981 and 1996 and for 13- to 20-year-old northern cod (NAFO Divisions 2J, 3K, and 3L) captured by commercial vessels between 1981 and 1992. The solid lines represent modelled relationships based on observed mean annual changes in weight at age x, i.e., w_{x+1}/w_x. The broken lines describe changes in weight at age at 50% of the observed growth rates. See text for more details.



mates of w_{x+1}/w_x were calculated in part from weights at age for cod aged 4–12 years measured during research surveys between 1981 and 1996 in NAFO Division 3L (Lilly 1997; and excluding weights at age based on sample sizes of less than 5). The comparatively few fish older than age 12 years sampled by the surveys necessitated the use of commercial catch statistics, combined for NAFO Divisions 2J, 3K, and 3L (1981–1992), to estimate growth rates for cod aged 13–20 years (Murphy et al. 1997). Although the latter data included weights of the slower-growing cod inhabiting the more northerly Divisions 2J and 3K, these do not seem to unduly affect the estimated growth rates for cod older than 12 years used in the present model (Fig. 1). Any bias in estimated individual growth rates for 3L cod introduced by the inclusion of cod from 2J and 3K is unlikely to be large, given the comparatively small influence of individual growth rate on population growth rate, documented later in this paper, and given the wide range in growth costs of reproduction considered in the present model (i.e., from observed to 50% reductions in annual changes to weight at

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Table 1. Age(x)-specific rates of weight (w) increase, i.e., w_{x+1}/w_x, for Atlantic cod aged 4–20 years.

Age-class (years)	Mean growth rate (w_{x+1}/w_x)	SD
4–5	1.62	0.20
5–6	1.43	0.17
6–7	1.30	0.16
7–8	1.28	0.23
8–9	1.26	0.19
9–10	1.21	0.50
10–11	1.10	0.51
11–12	1.26	0.44
12–13	1.21	0.11
13–14	1.21	0.14
14–15	1.25	0.16
15–16	1.11	0.13
16–17	1.30	0.35
17–18	1.24	0.39
18–19	0.94	0.13
19–20	1.08	0.28

Note: Data are means ± SD based on weights at age for cod aged 4–12 years measured during research surveys between 1981 and 1996 in NAFO Division 3L (minimum of five fish per sample; Lilly 1997) and based on weights at age for cod aged 13–20 years measured from the commercial catches of cod captured from NAFO Divisions 2J, 3K, and 3L (Murphy et al. 1997).

age). In addition, the range in individual growth rates considered here (the solid and broken lines in Fig. 1) encompass most of the observed weights at age measured from the survey and commercial catch data (Fig. 1). The error associated with individual increases in weight, ϵ_w, was assumed to be normally distributed with the standard deviation of each age-specific error distribution set equal to the standard deviation of the observed annual mean increases in weight at age (w_{x+1}/w_x) (Table 1).

Given the negative association between individual growth rate and age at maturity, θ, that has been repeatedly demonstrated in fish (Roff 1992; Hutchings 1993, 1996, and references therein), weights at age can be expected to differ among cod whose age at maturity ranges between 4 and 7 years (Murphy et al. 1997; Trippel et al. 1997). However, random samples of weights at age for cod maturing at ages 4, 5, 6, and 7 years are not available for northern cod (G.R. Lilly, Department of Fisheries and Oceans, P.O. Box 5667, St. John's, NF A1C 5X1, Canada, personal communication). To account for the high probability that cod maturing at ages 4–6 years will be the largest individuals at those ages (a supposition with empirical support for Georges Bank cod; Trippel et al. 1995), I calculated weight at maturity, w_θ, by incorporating both the observed age-specific incidence of maturity for northern cod (these data are unavailable for northern Grand Bank cod alone) and the normal distribution of weights at age, as determined from the research surveys (Murphy et al. 1997). Thus, for the present analysis, weights at maturity for ages $\theta = 4$, 5, and 6 years (there is a greater than 90% probability that cod aged 7 years are mature; Murphy et al. 1997) were randomly sampled from the upper p% of the normal distribution of weights for each age, where p% equals the observed incidence of maturity by age. The 1981–1996 average incidences of maturity were 1.5, 21.7, and 56.7% for $\theta = 4$, 5, and 6 years, respectively. Based on this formulation, the median weights at maturity of cod reproducing initially at ages 4, 5, and 6 years were 1.12, 1.55, and 2.06 kg, respectively. The weight of cod maturing at age 7 years was set equal to the observed average weight of 7-year-old cod measured during the research surveys between 1981 and 1996, i.e., 2.44 kg.

Growth cost of reproduction

The diversion of energy from somatic tissue production to the production of gonadal tissue effects a reduction in growth rate of reproductive individuals when compared with that of nonreproductive individuals (Bell 1980; Wootton 1990). The magnitude of this cost can range quite widely among populations of the same species. For example, in two populations of brook trout, *Salvelinus fontinalis*, on Cape Race, Newfoundland, the percentage reduction in annual growth rate experienced by maturing females was approximately equal to their gonadosomatic index (GSI) (gonad weight/total body weight) of roughly 10–15% (Hutchings 1993). At the other extreme, within another trout population on Cape Race (Freshwater River), for which individually marked fish were monitored for 4 years, annual increases in growth rate of reproductive individuals were 85% less than those of nonreproductive individuals of the same age (J.A. Hutchings, unpublished data).

There are comparatively few data on GSI for Atlantic cod. Estimates of average GSI for spawning female cod in the Northwest Atlantic in the early 1990s range from 0.08 in the southern Gulf of St. Lawrence (Schwalme and Chouinard 1999) to 0.19 in the northern Gulf (Lambert and Dutil 1997), giving a midrange GSI of 0.14. If the reduction in growth rate concomitant with maturation in cod can be approximated by the percentage of body tissue allocated to gonads, as it can for some populations of brook trout (Hutchings 1993), then the annual growth rates of reproductive cod would be about 14% less than those of nonreproductive cod at the same age. For cod maturing at age 7 years, I assumed that the observed weights at age from the survey and commercial catch data incorporated the reduction in growth rate effected by reproduction. For cod maturing at ages 4, 5, and 6 years, I multiplied their observed annual growth rates (w_{x+1}/w_x) by 0.86 until they reached age 7 years to simulate a 14% reduction in individual growth. Although the observed data include weights at age for both mature and immature fish, the incidence of maturity is comparatively low among 4- and 5-year-olds (1.5 and 21.7%, respectively, between 1981 and 1996), albeit increasing to 56.7% among 6-year-olds (Murphy et al. 1997). Thus, the incorporation of a growth cost of reproduction as applied here may underestimate the growth rate of cod maturing at age 6 years between ages 6 and 7 years. To examine how changes to this growth cost of reproduction influence population growth rate, I reduced observed growth rates (Table 1) by 10, 20, 30, 40, and 50%.

Prereproductive mortality and survival costs of reproduction

I partitioned the survival of cod into three categories: survival from birth to age 3 years (the age at which northern cod are first recruited to commercial fishing gear), instantaneous rate of mortality of immature individuals, Z_{imm}, and instantaneous rate of mortality of mature individuals, Z_{mat}.

Survival from birth to age 3 years was quantified from virtual population analysis estimates of age-specific abundance reported by Murphy et al. (1997) and from age-specific fecundities as defined above. Survival from birth to age 3 years in year t, $l_{3(t)}$, was assumed to be a function of the number n of 7- to 11-year-olds in year $t - 3$ multiplied by the average fecundity of those individuals, such that

$$(6) \qquad l_{3(t)} = n_{3(t)} \Big/ \sum_{x=7}^{x=11} n_{x(t-3)} m_{x(t-3)}.$$

Survival from birth to age 3 years estimated in this manner for the 1962–1988 year-classes of northern cod was weakly density de-

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Table 2. Survival parameter values used in the age-structured model of Atlantic cod life history.

Instantaneous mortality of immature females (Z_{imm})	Annual survival of immature females ($\exp(-Z_{imm})$)	Survival cost of reproduction ($1 - \gamma$)	Annual survival of mature females ($\exp(-Z_{imm})\gamma$)	Instantaneous mortality of mature females (Z_{mat})
0.10	0.91	0.10	0.82	0.20
		0.20	0.73	0.32
		0.30	0.64	0.45
0.20	0.82	0.10	0.74	0.30
		0.20	0.66	0.42
		0.30	0.57	0.55
0.30	0.74	0.10	0.67	0.40
		0.20	0.59	0.52
		0.30	0.52	0.65

pendent (see Myers et al. 1995) and averaged $1.13 \times 10^{-6} \pm 1.11 \times 10^{-6}$ (SD).

I assumed that variation in annual mortality attributable to factors unrelated to reproduction, e.g., predation, was best reflected by a beta distribution, given as

$$(7) \qquad f(x) = \frac{(x/s)^{(\alpha-1)}(1-(x/s))^{(\beta-1)}}{\Gamma(\alpha)\Gamma(\beta)/\Gamma(\alpha+\beta)}$$

where $\Gamma(\beta)$ is the Gamma function. With the scale parameter, s, establishing the upper bound of the distribution at 1.0 and the mean of the distribution ($\alpha/(\alpha + \beta)$) set to annual survival probabilities, i.e., $\exp(-Z_{imm})$ of 0.91, 0.82, and 0.74 (corresponding to Z_{imm} of 0.1, 0.2, and 0.3, respectively), survival was approximated by a negatively skewed distribution. The rationale for selecting a beta distribution to model survival data is based on its simplicity, smoothness, and flexibility, making it an ideal choice for distributions that have restricted support, i.e., whose range is limited, in this case between 0 and 1 (Johnson et al. 1994).

Survival from birth to age x, i.e., l_x, will differ between reproductive and nonreproductive individuals. The survival costs of reproduction experienced by reproductive cod may be attributable to factors such as the energetic consequences of producing gonadal tissue (Lambert and Dutil 1997), the repertoire of behaviours involved in mate competition and possibly mate choice (Brawn 1961; Hutchings et al. 1999), and a cessation of feeding often concomitant with breeding (Fordham and Trippel 1999). I assume here a cost ($1 - \gamma$) of 0.10, such that survival from age x to age $x + 1$ for an individual reproducing at age x is

$$(8) \qquad l_{x+1} = l_x \exp(-Z_{imm})\ \gamma.$$

The influence of higher survival costs on population growth rate was examined by reducing γ from 0.9 to 0.8 and to 0.7. The full range of parameter values for survival used here is given in Table 2.

Results

Influence of age-specific survival and weight on maximum population growth rate (r)

For the same survival probabilities and growth costs of reproduction, rates of increase of early-maturing Atlantic cod ($\theta = 4$ years) were consistently greater than those of late-maturing cod ($\theta = 7$ years) (compare Figs. 2A–2C with Figs. 2D–2F). At low prereproductive mortality ($Z_{imm} = 0.10$), the annual rate of increase of cod maturing at age 4 years was about 2–4% higher than that of cod maturing at 7 years. This difference in r between early- and late-maturing cod increased with increases in prereproductive mortality, Z_{imm} (Fig. 2). The highest estimates of r for cod maturing at ages 4 and 7 years were 0.164 and 0.135, respectively, corresponding to maximum rates of increase of 18 and 14% per annum.

Population rates of increase were particularly sensitive to changes in survival prior to and after maturity (Fig. 2). All else being equal, an increase in Z_{imm} of 0.1 was associated with a reduction in r of about 0.03–0.05. Similarly, 10% increases in survival costs of reproduction (reflected by the incremental increases in Z_{mat} on Fig. 2) were associated with declines in r of about 0.03 to 0.04, independent of age at maturity. Particularly large increases in prereproductive mortality from $Z_{imm} = 0.1$ to 0.3, especially when coupled with increases in survival costs of reproduction, often resulted in negligible or negative population growth (e.g., Figs. 2C, 2E, and 2F).

By contrast, changes to individual growth rate, as reflected by increased growth costs of reproduction, had comparatively little effect on population rates of increase (Fig. 2). Linear regressions relating r to reductions in individual growth rate (Table 3) for the values of pre- and postreproductive mortality used here revealed a similarity in slopes, specific to age at maturity, such that a 10% reduction in postreproductive individual growth rate was predicted to reduce r by only 0.006 and 0.003 (the averages of the regression slopes) for individuals maturing at 4 and 7 years, respectively. At most, all else being equal, declines in postreproductive growth rate of up to 50% of observed values were associated with reductions in r of only about 0.03 (Fig. 2).

Probabilities of negative population growth

The frequency distributions of R_0 produced by the stochastic population growth model (e.g., Fig. 3) can be used to estimate the probabilities with which various rates of population growth rate can be achieved under specific combinations of age at maturity, survival and growth costs of reproduction, and individual postreproductive growth rate. These distributions were used to estimate the probabilities of northern Grand Bank cod experiencing growth rates that would allow the population to more than double (i.e., $R_0 > 2$) or to decline during a single generation (i.e., $R_0 < 1$). Generation times, which vary with age-specific survival and fecundity (see eq. 4), ranged between 5.4 and 11.4 years for cod maturing at age 4 years and between 8.3 and 12.9 years for cod maturing at age 7 years (Table 4).

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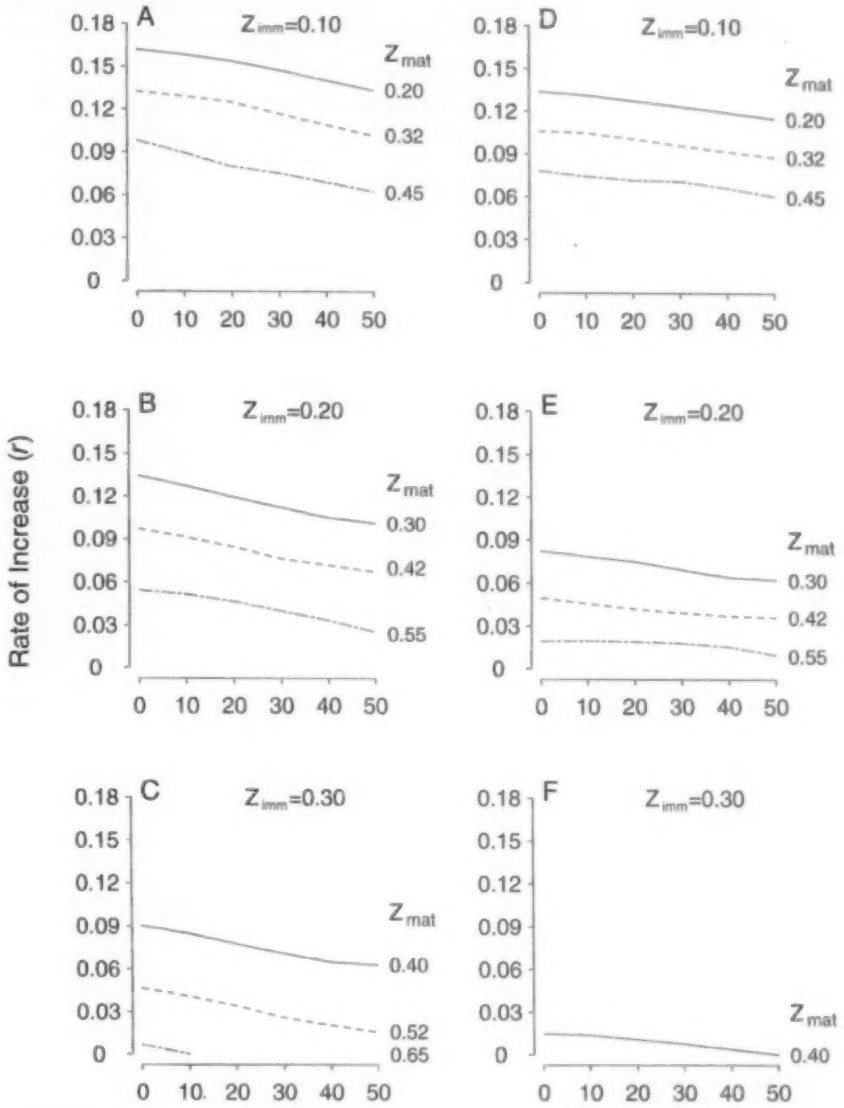
Fig. 2. Changes in maximum Atlantic cod population growth rate, r, with changes in age at maturity of (A–C) 4 years and (D–F) 7 years, prereproductive mortality (Z_{imm}), postreproductive mortality (Z_{mat}) effected by incremental declines in survival probabilities, and individual postreproductive growth rate.



Although the probability of a per-generation doubling in population size declined with decreases in postreproductive growth rate and with increases in age at maturity, their influence on $Pr(R_0 > 2)$ was comparatively small when compared with that of changes in survival (Fig. 4). At observed weights at age (i.e., 0% decline in observed postreproductive growth rate) and at the survival probabilities assumed to be typical for this stock (i.e., $Z_{imm} = 0.1$; $Z_{mat} = 0.2$, e.g., Pinhorn 1975), the probability of northern Grand Bank cod doubling every generation, at population sizes comparatively uninfluenced by density, was about 0.84 (the average of the probabilities for $\theta = 4$ and 7 years; Figs. 4A and 4D). Incremental increases in prereproductive mortality, Z_{imm}, from 0.1 to 0.2 and to 0.3 were associated with incremental declines in $Pr(R_0 > 2)$ of 10–20%. Similar incremental reductions in $Pr(R_0 > 2)$ were associated with incremental 10% declines in postreproductive mortality.

The probability of negative population growth between generations, i.e., $Pr(R_0 < 1)$, was also comparatively uninfluenced either by individual growth rate or by age at maturity (Fig. 5). At observed weights at age and at presumed typical mortalities of Z_{imm} and Z_{mat} of 0.1 and 0.2, respectively, the

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Table 3. Linear regressions between Atlantic cod population growth rate (r) and proportional reduction (G) in annual individual growth rate (w_{x+1}/w_x) between ages x and $x + 1$ for ages at maturity (θ) of 4 and 7 years and for different instantaneous rates of mortality prior to (Z_{imm}) and following (Z_{mat}) maturity.

θ	Z_{imm}	Z_{mat}		R^2	p
4	0.10	0.20	$r = -0.058G + 0.164$	0.97	<0.001
		0.32	$r = -0.066G + 0.136$	0.95	<0.001
		0.45	$r = -0.065G + 0.094$	0.96	<0.001
	0.20	0.30	$r = -0.071G + 0.134$	0.96	<0.001
		0.42	$r = -0.066G + 0.097$	0.97	<0.001
		0.55	$r = -0.060G + 0.057$	0.90	0.004
	0.30	0.40	$r = -0.061G + 0.091$	0.89	0.004
		0.52	$r = -0.068G + 0.048$	0.96	<0.001
7	0.10	0.20	$r = -0.039G + 0.135$	0.98	<0.001
		0.32	$r = -0.039G + 0.108$	0.95	<0.001
		0.45	$r = -0.027G + 0.078$	0.66	0.049
	0.20	0.30	$r = -0.043G + 0.082$	0.94	0.002
		0.42	$r = -0.028G + 0.049$	0.94	0.001
		0.55	$r = -0.017G + 0.022$	0.43	0.157
	0.30	0.40	$r = -0.032G + 0.017$	0.94	0.002

Fig. 3. Frequency distributions of net reproductive rate, R_0, as determined from 2000 iterations of a stochastic, age-structured model of population growth for Atlantic cod maturing at ages 4 and 7 years at two instantaneous rates of postreproductive mortality (Z_{mat}). The black vertical lines correspond to the net reproductive rate at which individuals are replacing themselves between generations, i.e., $R_0 = 1$. The proportion of the frequency distributions to the right of the black lines (grey lines) represents the probability that the population will grow between generations. Prereproductive mortality is held constant at $Z_{imm} = 0.1$.

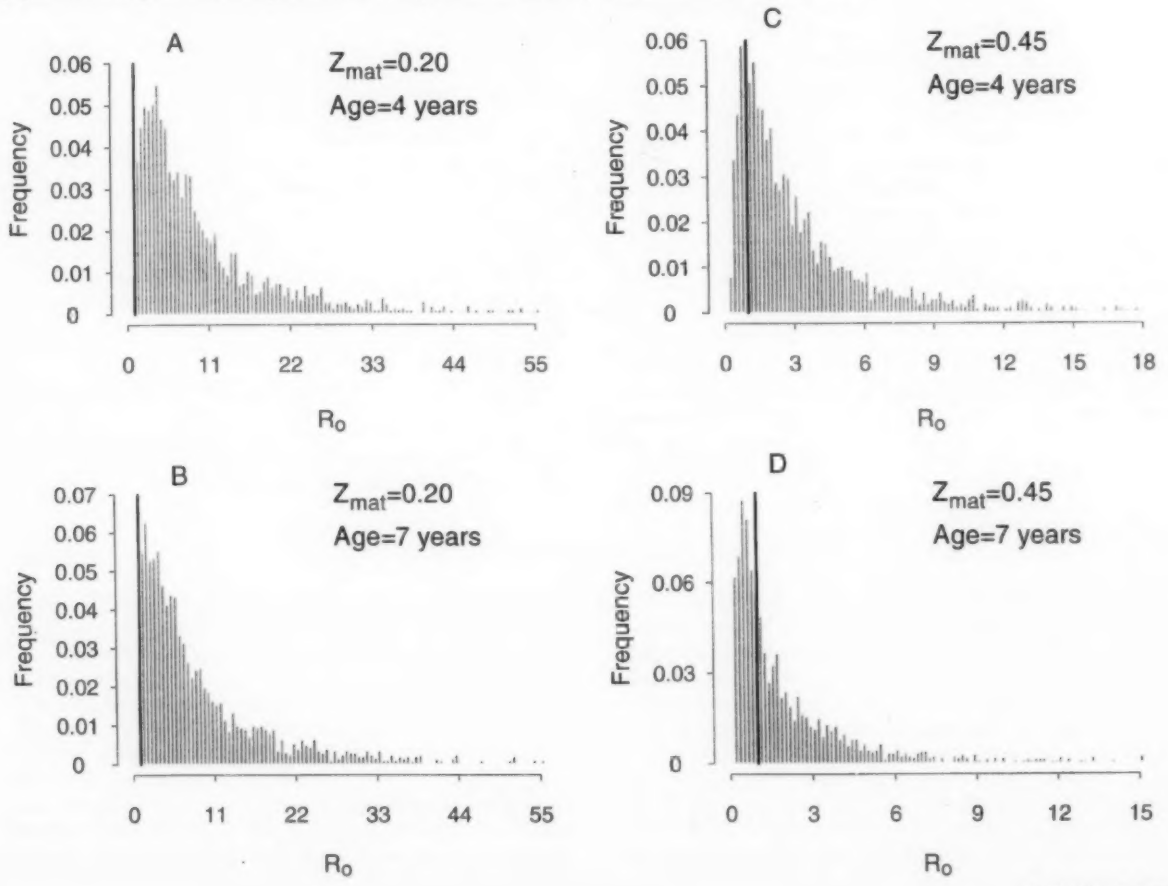

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Table 4. Deterministic estimates of maximum population growth rate (r, R_0) and generation time (G) for Atlantic cod maturing at ages 4 and 7 years at observed and 50% of observed postreproductive growth rates and at different levels of prereproductive mortality (Z_{imm}) and postreproductive mortality (Z_{mat}).

			Age at maturity = 4 years			Age at maturity = 7 years		
Z_{imm}	Z_{mat}	Postreproductive growth rate	r	R_0	G (years)	r	R_0	G (years)
0.10	0.20	Observed	0.204	10.14	11.36	0.174	9.36	12.89
		50% decline	0.178	5.34	9.40	0.154	6.01	11.66
	0.32	Observed	0.178	4.94	8.95	0.147	5.19	11.19
		50% decline	0.154	3.18	7.53	0.130	3.79	10.24
	0.45	Observed	0.150	2.91	7.12	0.122	3.28	9.77
		50% decline	0.123	2.17	6.29	0.107	2.66	9.17
0.20	0.30	Observed	0.171	4.88	9.27	0.116	3.74	11.41
		50% decline	0.144	3.06	7.76	0.095	2.69	10.42
	0.42	Observed	0.144	2.94	7.48	0.088	2.42	10.07
		50% decline	0.116	2.31	6.53	0.069	1.92	9.39
	0.55	Observed	0.111	2.01	6.29	0.060	1.73	9.07
		50% decline	0.083	1.61	5.74	0.044	1.47	8.66
0.30	0.40	Observed	0.135	2.82	7.69	0.052	1.70	10.23
		50% decline	0.105	2.01	6.67	0.030	1.33	9.51
	0.52	Observed	0.104	1.97	6.52	0.023	1.24	9.27
		50% decline	0.073	1.54	5.89	0.004	1.04	8.81
	0.65	Observed	0.069	1.49	5.75	–0.005	0.96	8.59
		50% decline	0.042	1.25	5.37	–0.020	0.85	8.3

probability of northern Grand Bank cod declining during a single generation was less than 5% (Figs. 5A and 5D). However, $Pr(R_0 < 1)$ increased by about 10–20% for every 0.1 incremental increase in Z_{imm} and for every 0.1 reduction in postreproductive survival probability.

The stochastic estimates of population growth (e.g., Fig. 3) were consistently less than those estimated by the deterministic model, in which rates of survival and fecundity were assumed invariant within age (Table 4). Varying with pre- and post-reproductive mortality and with growth costs of reproduction, deterministic estimates of r generally exceeded their corresponding stochastic estimates by 0.04–0.06. At assumed typical pre- and post-reproductive mortalities of $Z_{imm} = 0.1$ and $Z_{mat} = 0.2$, differences between deterministic (0.204 and 0.174 at $\theta = 4$ and 7 years, respectively) and stochastic (0.164 and 0.135 at $\theta = 4$ and 7 years, respectively) estimates of r corresponded to a 5% difference in annual rate of increase.

Discussion

Maximum rates of population increase for northern Atlantic cod

The present study describes how age at maturity, individual growth rate, and pre- and post-reproductive survival can influence metrics of population increase for Atlantic cod. It differs from previous efforts to incorporate reproductive costs in life history analyses of this species (e.g., Myers and Doyle 1983; Trippel et al. 1995) by attempting to disentangle the effects of individual age, growth, and mortality on maximum population growth rate. Based on an age-structured life history model, and incorporating stochastic variation in survival and fecundity (the latter through changes in weight at age), annual (r) and per-generation (R_0) rates of population increase appeared to be most sensitive to changes in pre- and post-reproductive survival probabilities and less sensitive to changes in age at maturity and post-reproductive growth rate.

At observed weights at age and at the instantaneous rate of natural mortality generally assumed typical for northwest Atlantic cod, i.e., $M = 0.2$, or $Z = 0.2$ in the absence of fishing, median estimates of r for ages at maturity of 4–7 years were estimated here to range between 0.135 and 0.164. These estimates, coupled with that of 0.26 previously estimated for northern cod (Myers et al. 1997*a*), correspond to a range of maximum annual rates of increase of 14–29% and may provide a reasonable approximation of the typical range in r for Atlantic cod inhabiting the waters off northern and eastern Newfoundland.

Influence of increased mortality on population growth rate

The present study suggests that changes in mortality may have a larger effect on population rate of increase than changes to individual growth rate or age at maturity. Significant reductions in r were associated with increases in either pre- or post-reproductive mortality, although the effect on r generated by changes to Z_{imm} was greater for cod maturing at 7 rather than at 4 years of age (Fig. 2). This can be attributed to the greater number of years experienced by late-maturing cod prior to initial reproduction. At observed weights at age and at a prereproductive mortality of $Z_{imm} = 0.1$, increases in Z_{mat} from 0.20 to 0.45 are predicted to reduce r from 0.162 to 0.098 for cod maturing at age 4 years and from 0.134 to 0.079 for cod maturing at 7 years.

If the M of some Atlantic cod stocks has increased since the commercial fishery closures in the early 1990s (DFO 1998*a*, 1998*b*), and if an increase in M (effectively Z in the absence of fishing) from 0.2 to 0.4 is a reasonable approximation of the magnitude of this change (Sinclair 1999), the negative consequences to population growth rate may be substantial. The present study suggests that an increase in

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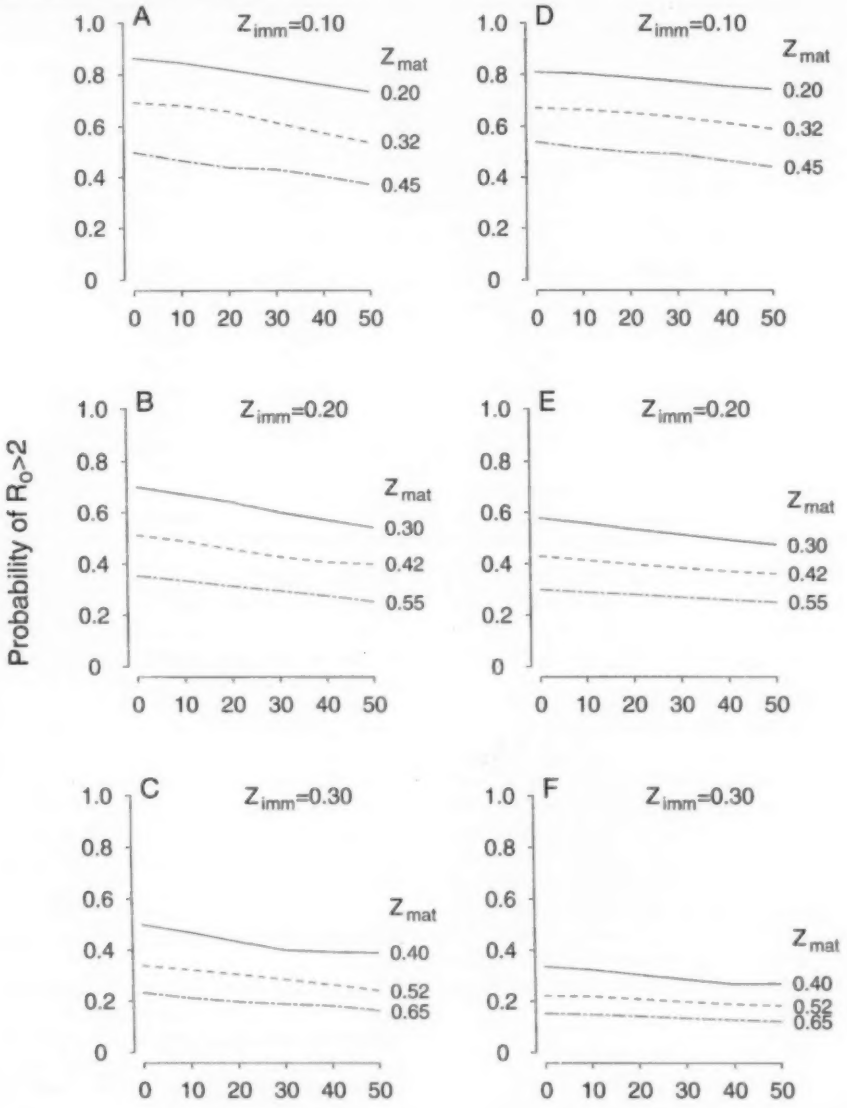
Fig. 4. Probability of Atlantic cod more than doubling in population size within a single generation, $Pr(R_0 > 2)$ as a function of age at maturity of (A–C) 4 years and (D–F) 7 years, prereproductive mortality (Z_{imm}), postreproductive mortality (Z_{mat}), and individual growth rate. Generation times are given in Table 4.



Z_{mat} from 0.20 to 0.45, in the absence of changes to weights at age and to prereproductive mortality, will reduce the annual growth rate of northern Grand Bank cod from between 14 and 18% to between 8 and 10% per annum. In terms of doubling time, i.e., $\ln(2)/r$, such an increase in mortality would effect increases in doubling time from 4.3 to 7.1 years for cod maturing at 4 years and from 5.2 to 8.8 years for cod maturing at 7 years.

Age at maturity and population growth

There is evidence to suggest that age at maturity for some Atlantic cod stocks may have decreased during the past decade (Murphy et al. 1997; Trippel et al. 1997). Rapid declines in age at maturity in fishes are usually associated with increasing growth rates prior to first reproduction (Roff 1992; Stearns 1992; Hutchings 1993, 1996). Such a negative association between growth rate and age at maturity has, for example, been documented for Atlantic cod on Georges Bank (Trippel et al. 1995). However, evidence of such an increase in individual growth rate is not apparent from recent research survey estimates of weights at age for Northwest Atlantic cod (e.g., Trippel et al. 1997). Increases in body

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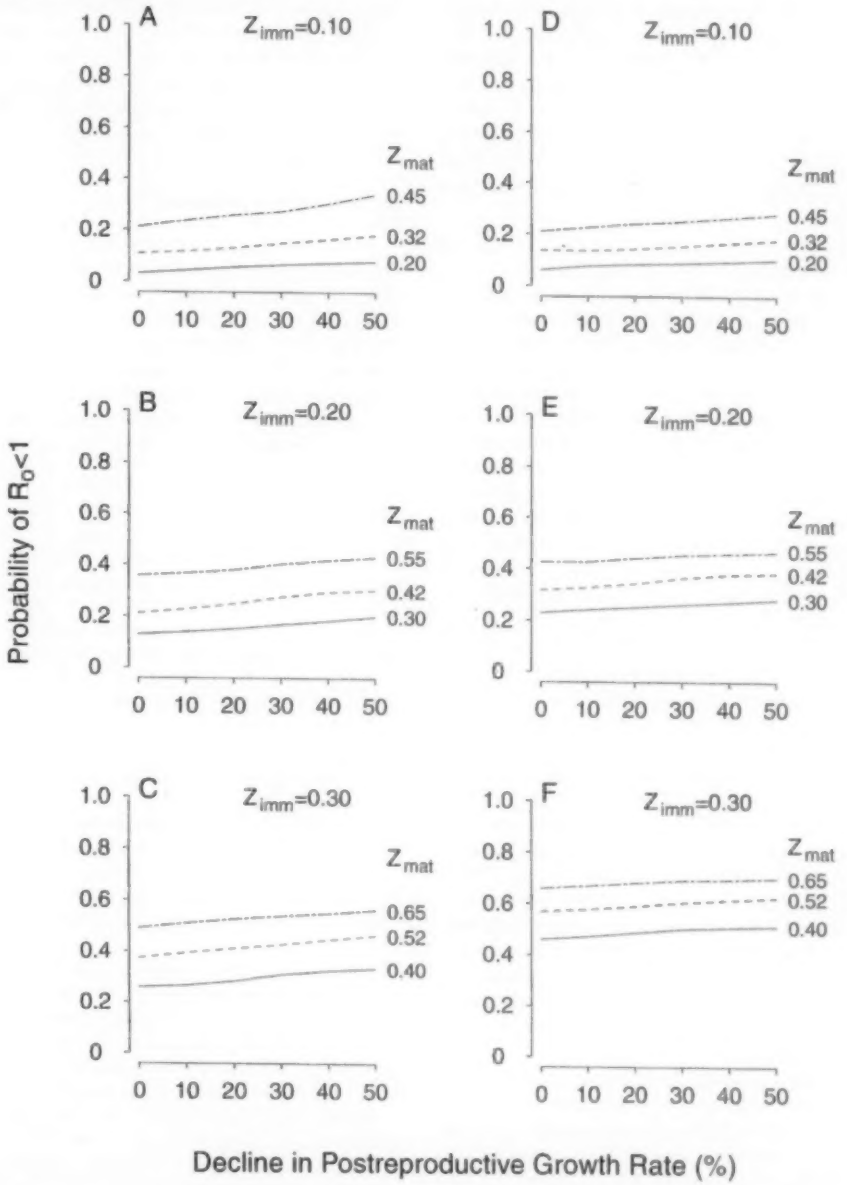
Fig. 5. Probability of negative population growth within a single generation, $Pr(R_0 < 1)$, as a function of age at maturity of (A–C) 4 years and (D–F) 7 years, prereproductive mortality (Z_{imm}), postreproductive mortality (Z_{mat}), and individual growth rate. Generation times are given in Table 4.



condition can also effect reduced ages at maturity (Stearns 1992). But, again, the apparent declines in age at maturity have not been associated with appreciable increases in body condition (e.g., Lilly 1997).

A third explanation for declining age at maturity is differential loss of genotypes from the population. Assuming that age at maturity is heritable in Atlantic cod, as it is in most organisms (Roff 1997), a decline in age at maturity may have been caused by an increase in the proportional representation of early-maturing genotypes predicated by the higher fishing mortality experienced by the comparatively large, late-maturing genotypes in the late 1980s and early 1990s (Hutchings and Myers 1994; Myers et al. 1997*b*; Lilly et al. 1998).

Nonetheless, irrespective of what may have caused a decline in θ, a decline in age at maturity would appear, all else being equal, to have a positive, although comparatively small, effect on population growth rate (Fig. 2). This result may, however, be misleading. The estimates of r in the present analyses, incorporating the well-documented effect of individual growth rate on age at maturity, were based on the assumption that cod maturing relatively early in life were

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the fastest growers in the population. This resulted in comparatively larger weights at age and correspondingly higher age-specific fecundities for early-maturing individuals. If, however, early-maturing genotypes share the same age-specific schedule of weight as late-maturing genotypes, the analyses of r presented here will overestimate the intrinsic rate of increase of early-maturing genotypes. Additional factors that may reduce the fitness of comparatively small, early-maturing individuals may include reduced hatching success (Trippel 1998), reduced mating success (Hutchings et al. 1999), and increased recruitment variability (Hutchings and Myers 1993).

Weaknesses and strengths of the present model

The weaknesses of the present model ultimately lie in the empirical bases, or lack thereof, of the model parameters. The variances used in the error distributions are, with the exception of those assumed for the mortality experienced by cod older than 3 years, empirically based on virtual population analysis estimates of abundance (to quantify survival from birth to age 3 years), research survey and commercial catch estimates of weights at age, and a very broadly based fecundity–weight relationship. Clearly, these estimates can be improved upon. For example, there is a need to construct detailed fecundity – body size regressions for Atlantic cod throughout its range (e.g., see Pinhorn 1984), given that population differences in fecundity per unit weight will generate population differences in rate of increase.

Additional limitations of the present analysis lie in the assumptions that fecundity is independent of parity and that the probability of surviving from birth to age 3 years is independent of parental age or body size. Trippel (1998), for example, documented hatching rates of virgin cod that were 25% those of previously spawned females. Presumably, such a decline in hatching success would be evident irrespective of age at maturity, although such an experiment has yet to be undertaken. An effect of age and body size on cod reproductive success was suggested by Hutchings and Myers (1993) who posited that the shorter spawning period expected of younger, smaller females may increase recruitment variability because of a reduced probability of temporal overlap between periods of phytoplankton blooms and larval cod feeding. The present model also includes the assumption that cod spawn annually. If they do not, population growth rates will be negatively affected.

Perhaps the primary advantage of using a stochastic life history model to estimate rates of population increase lies in its potential to account for empirically reasonable variation in the parameters and variables on which rates of increase are based. For example, depending on the magnitude of survival and growth reproductive costs, the present study suggests that the probability of very slow or even negative growth can be significant for Atlantic cod. This is consistent with the findings of Lewontin and Cohen (1969) and Roff (1974) that random variation in population growth rate can have seriously destabilizing effects on population dynamics, even though the expected population size may increase with time. Also, the effect of age-specific changes to survival and individual growth on population growth rate cannot be readily quantified by other means of estimating r, e.g., stock–recruitment relationships (Myers et al. 1997*a*). In addition, stochastic models provide probabilities with which specific population rates of increase might be realized. As examples, I selected the probabilities with which a population will decline and double every generation. Such probabilities may prove useful in risk analyses.

The use of stochastic, age-structured life history models in fish stock assessment and fish conservation biology appears to have merit. The strengths and weaknesses of such an approach warrant further examination.

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References

Bell, G. 1980. The costs of reproduction and their consequences. Am. Nat. **116**: 45–76.

Bell, G. 1997. The basis of selection. Chapman and Hall, New York.

Brawn, V.M. 1961. Reproductive behaviour of the cod (*Gadus callarias* L.). Behaviour, **18**: 177–197.

Charlesworth, B. 1994. Evolution in age-structured populations. Cambridge University Press, Cambridge, U.K.

DFO. 1998*a*. Northern (2J3KL) cod. Stock Status Report A2-01 (1998). Department of Fisheries and Oceans, Science Branch, St. John's, Nfld.

DFO. 1998*b*. Cod in the southern Gulf of St. Lawrence. Stock Status Report A3-01 (1998). Department of Fisheries and Oceans, Science Branch, Moncton, N.B.

Fordham, S.E., and Trippel, E.A. 1999. Feeding behaviour of cod (*Gadus morhua*) in relation to spawning. J. Appl. Ichthyol. **15**: 1–9.

Hutchings, J.A. 1993. Adaptive life histories effected by age-specific survival and growth rate. Ecology, **74**: 673–684.

Hutchings, J.A. 1996. Adaptive phenotypic plasticity in brook trout, *Salvelinus fontinalis*, life histories. Ecoscience, **3**: 25–32.

Hutchings, J.A., and Myers, R.A. 1993. The effect of age on the seasonality of maturation and spawning of Atlantic cod, *Gadus morhua*. Can. J. Fish. Aquat. Sci. **50**: 2468–2474.

Hutchings, J.A., and Myers, R.A. 1994. What can be learned from the collapse of a renewable resource? Atlantic cod, *Gadus morhua*, of Newfoundland and Labrador. Can. J. Fish. Aquat. Sci. **51**: 2126–2146.

Hutchings, J.A., Bishop, T.D., and McGregor-Shaw, C.R. 1999. Spawning behaviour of Atlantic cod, *Gadus morhua*: evidence of mate competition and mate choice in a broadcast spawner. Can. J. Fish. Aquat. Sci. **56**: 97–104.

Johnson, N.L., Kotz, S., and Balakrishnan, N. 1994. Continuous univariate distributions. John Wiley & Sons, New York.

Lambert, Y., and Dutil, J.-D. 1997. Condition and energy reserves of Atlantic cod (*Gadus morhua*) during the collapse of the northern Gulf of St. Lawrence stock. Can. J. Fish. Aquat. Sci. **54**: 2388–2400.

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Lande, R. 1993. Risks of population extinction from demographic and environmental stochasticity and random catastrophes. Am. Nat. **142**: 911–927.

Lewontin, R.C., and Cohen, D. 1969. On population growth in a randomly varying environment. Proc. Natl. Acad. Sci. U.S.A. **62**: 1056–1060.

Lilly, G.R. 1997. Size and condition of cod in Divisions 2J+3KL during 1978–1996. NAFO SCR Doc. 97/62.

Lilly, G.R., Shelton, P.A., Brattey, J., Cadigan, N., Murphy, E.F., Stansbury, D.E., Davis, M.B., and Morgan, M.J. 1998. An assessment of the cod stock in NAFO Divisions 2J+3KL. Can. Stock Assess. Secret. Res. Doc. 98/15.

May, A.W. 1967. Fecundity of Atlantic cod. J. Fish. Res. Board Can. **24**: 1531–1551.

Murphy, E.F., Stansbury, D.E., Shelton, P.A., Brattey, J., and Lilly, G.R. 1997. A stock status update for NAFO Divisions 2J+3KL cod. NAFO SCR Doc. 97/59.

Myers, R.A., and Doyle, R.W. 1983. Predicting natural mortality rates and reproduction — mortality trade-offs from fish life history data. Can. J. Fish. Aquat. Sci. **40**: 612–620.

Myers, R.A., Barrowman, N.J., Hutchings, J.A., and Rosenberg, A.A. 1995. Population dynamics of exploited fish stocks at low population levels. Science (Washington, D.C.), **269**: 1106–1108.

Myers, R.A., Mertz, G., and Fowlow, P.S. 1997*a*. Maximum population growth rates and recovery times for Atlantic cod, *Gadus morhua*. Fish. Bull. U.S. **95**: 762–772.

Myers, R.A., Hutchings, J.A., and Barrowman, N.J. 1997*b*. Why do fish stocks collapse? The example of cod in Atlantic Canada. Ecol. Appl. **7**: 91–106.

Pinhorn, A.T. 1975. Estimates of natural mortality of the cod stock complex in ICNAF Divisions 2J, 3K and 3L. Int. Comm. Northwest Atl. Fish. Res. Bull. **11**: 31–36.

Pinhorn, A.T. 1984. Temporal and spatial variation in fecundity of Atlantic cod (*Gadus morhua*) in Newfoundland waters. J. Northwest Atl. Fish. Sci. **5**: 161–170.

Roff, D.A. 1974. The analysis of a population model demonstrating the importance of dispersal in a heterogeneous environment. Oecologia, **15**: 259–275.

Roff, D.A. 1992. The evolution of life histories: theory and analysis. Chapman and Hall, New York.

Roff, D.A. 1997. Quantitative evolutionary genetics. Chapman and Hall, New York.

Schwalme, K., and Chouinard, G.A. 1999. Seasonal dynamics in feeding, organ weights, and reproductive maturation of Atlantic cod (*Gadus morhua* L.) in the southern Gulf of St. Lawrence. ICES J. Mar. Sci. **56**: 303–319.

Sinclair, A.F. 1999. Natural mortality of cod in the southern Gulf of St. Lawrence. ICES J. Mar. Sci. In press.

Stearns, S.C. 1992. The evolution of life histories. Cambridge University Press, Cambridge, U.K.

Trippel, E.A. 1998. Egg size, viability and seasonal offspring production of young Atlantic cod. Trans. Am. Fish. Soc. **127**: 339–359.

Trippel, E.A., Buzeta, M.-I., and Hunt, J.J. 1995. Evaluation of the cost of reproduction in Georges Bank Atlantic cod (*Gadus morhua*): utility of otolith back-calculation. *In* Recent developments in fish otolith research. *Edited by* D.H. Secor, J.M. Dean, and S.E. Campana. University of South Carolina Press, Columbia, S.C. pp. 599–610.

Trippel, E.A., Morgan, M.J., Fréchet, A., Rollet, C., Sinclair, A., Annand, C., Beanlands, D., and Brown, L. 1997. Changes in age and length at sexual maturity of northwest Atlantic cod, haddock and pollock stocks, 1972–1995. Can. Tech. Rep. Fish. Aquat. Sci. No. 2157.

Wootton, R.J. 1990. Ecology of teleost fishes. Chapman and Hall, London.

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Cumulative effects of multiple effluent and low dissolved oxygen stressors on mayflies at cold temperatures

Richard B. Lowell and Joseph M. Culp

Abstract: Effluents produced by pulp mills and sewage plants on northern rivers have the potential for a variety of interacting effects on downstream benthic invertebrates via increased levels of toxicants and nutrients and decreased levels of dissolved oxygen (DO) in the substratum. We experimentally measured the combined effects of these stressors at low temperature (4.5°C) on a common northern river invertebrate, the mayfly *Baetis tricaudatus* Dodds. Mayflies were exposed in laboratory artificial streams to one of two DO levels (low (5 $mg \cdot L^{-1}$) versus high (11 $mg \cdot L^{-1}$)) in the presence and absence of pulp mill and sewage effluent from an Alberta, Canada, mixed-effluent outfall (control river water versus 1% effluent); the DO and effluent treatments bracketed typical field concentrations. In the low-DO treatment, grazing intensity was reduced by 80%, and after 2 weeks of exposure, survival was reduced by 60–90%. Furthermore, 250–350% more mayflies in the low-DO treatment moved upward into regions of greater current velocity close to the surface of the artificial streams, a behavior that would likely make them more susceptible to fish predation in the field. In contrast, the 1% effluent treatment increased mayfly survival (possibly due, in part, to stimulation of increased mayfly grazing intensity by the effluent), although this effect only partly compensated for the pronounced negative impact of low DO levels.

Résumé : Les effluents des usines de pâtes et des stations d'épuration des eaux usées rejetés dans les cours d'eau nordiques peuvent avoir différents effets interdépendants sur les invertébrés benthiques en aval à cause de la hausse des concentrations de produits toxiques et de substances nutritives et de la diminution des concentrations d'oxygène dissous (OD) dans le substrat. Nous avons mesuré expérimentalement les effets combinés de ces facteurs de stress à basse température (4,5°C) chez l'éphémère *Baetis tricaudatus* Dodds, un invertébré commun des cours d'eau nordiques. Les éphémères ont été exposées, dans des cours d'eau artificiels en laboratoire, à deux concentrations différentes d'oxygène dissous (faible (5 $mg \cdot L^{-1}$) vs. élevée (11 $mg \cdot L^{-1}$)) en présence et en l'absence d'effluents d'usine de pâtes et de station d'épuration provenant d'un exutoire d'effluents mixtes situé en Alberta (Canada) (eau d'une rivière témoin et 1 % d'effluents); l'expérience en présence d'oxygène dissous et d'effluents a regroupé des concentrations caractéristiques mesurées sur le terrain. En présence d'une faible concentration d'oxygène dissous, l'intensité du broutage a été réduite de 80 % et, après deux semaines d'exposition, la survie était réduite de 60 à 90 %. De plus, nous avons noté que les éphémères mises en présence d'une faible quantité d'oxygène dissous étaient de 250 à 350 % plus nombreuses à se déplacer vers l'amont, où la vitesse du courant est plus élevée près de la surface des cours d'eau artificiels, comportement qui les rendrait probablement plus vulnérables à la prédation exercée par les poissons dans la nature. Par contraste, l'exposition à 1% d'effluents a augmenté la survie des éphémères (ce qui peut être causé, en partie, par le fait que l'effluent stimule l'intensité du broutage), bien que cet effet ne compense qu'en partie les effets négatifs marqués des faibles concentrations d'oxygène dissous.

[Traduit par la Rédaction]

Introduction

Effluents from pulp mills and municipal sewage plants are the major point sources of toxicant and nutrient loading in many of Canada's large river systems. Toxicants in the effluents have the potential for negative impacts on benthic invertebrates in these rivers (Servos et al. 1996). Pulp mill effluents, in particular, typically contain numerous toxicants, including metals, fatty acids, and chlorinated and non-chlorinated organic compounds, as well as hundreds of unidentified organic compounds. In contrast, the nutrients found in these effluents can stimulate increased algal and microbial growth rates, which, in turn, may affect the abundance and size distribution of benthic invertebrate herbivores (Chambers 1996; Culp and Podemski 1996). Benthic invertebrates may be further affected when effluents contribute to lowered levels of dissolved oxygen (DO) in the substratum where these organisms are found.

Effluent-induced depressions of DO levels have been well documented in more southerly temperate rivers, especially at high summer temperatures (Davis 1975; Chambers et al. 1997). Low DO levels can, however, also be quite widespread in more northerly rivers (e.g., Alaska, Canada, Rus-

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sia; Schallock and Lotspeich 1974; Chambers et al. 1997). For example, low DO levels can occur in the water column of northern rivers such as the Athabasca River in Alberta, Canada. In this river, DO levels vary substantially through the year, with the lowest water column levels being observed downriver of pulp mill and municipal effluent outfalls during the winter when ice-cover restricts reaeration of oxygen-depleted waters (Chambers et al. 1997).

Of particular relevance to benthic invertebrates, effluent can also contribute to lowered DO levels within the substratum of cold-water rivers, even when water column DO is near saturation levels. For example, in northern Alberta in the spring, we have measured DO level reductions of as much as 8–9 mg·L^{-1} (3–4 mg·L^{-1} in the substratum versus 12 mg·L^{-1} in the water column) in the upper 10 cm of gravel–cobble substratum 50 km downriver of pulp mill and sewage effluent outfalls at temperatures ≤5°C as compared with upstream areas sampled at the same time where intrasubstratum DO was typically within 2–3 mg·L^{-1} of the near-saturation water column levels (unpublished data).

Although some data are available in Canada and elsewhere on the effects of low DO levels on warmer water freshwater invertebrates (e.g., Nebeker 1972; Chapman 1986; Winter et al. 1996), information on the oxygen requirements of invertebrates found in cold-water northern rivers is very limited. In addition, the data that have been collected for warmer water species come mostly from short-term exposures of less than 1 week (reviewed in Davis 1975; Chapman 1986). Furthermore, very little data are available to assess the combined effects of pulp mill and municipal effluent and low levels of DO on aquatic organisms of any kind. Previous work with bleached kraft pulp mill effluent or its constituents in the United States (Hicks and DeWitt 1971; Graves et al. 1981) and Canada (Kruzynski 1979; see also Birtwell 1989) 20–30 years ago showed that contaminant toxicity to fish increased with decreasing DO levels. Over the last 20 years, however, pulp mill effluent treatment has improved markedly, significantly reducing incidents of acute toxicity to fish and invertebrates in North America, although further work is needed on longer-term effects on survival and sublethal parameters, such as behavior (Servos et al. 1996). Therefore, additional information is required to determine the overall effect on riverine biota, including benthic invertebrates, of the pulp mill effluent presently being released into North American rivers, particularly in northern regions where low temperatures are common through most of the year.

Benthic invertebrates are a major source of food for northern riverine fishes. For many of these invertebrates, the cold-water period spans much of their life history. Thus, effluent and oxygen levels during the lengthy cold-water period likely play a major role in invertebrate growth and, ultimately, fitness. Therefore, regulating pulp mill and sewage effluents to manage fisheries resources and protect northern aquatic ecosystems requires answers to the following questions. (*i*) What are the combined effects of pulp mill and sewage effluents on benthic invertebrates under cold-water temperature regimes? (*ii*) Do the negative toxicant or the growth-related nutrient enhancement effects predominate at low temperatures? (*iii*) What is the added effect of the low levels of DO experienced by northern river invertebrates in the substratum at low temperatures? The research reported here begins to address these complex questions via experimental manipulations of cold-water low DO levels and pulp mill and sewage effluent and measurements of their combined impacts on the grazing intensity, microhabitat positioning, and survival of a common northern river invertebrate, the mayfly *Baetis tricaudatus* Dodds.

Materials and methods

Experimental design

Exposure of *B. tricaudatus* to effluent and DO was controlled by conducting experiments in artificial streams arranged in a 2 × 2 factorial design. The mayflies were exposed to one of two levels of DO (low (5 mg·L^{-1}) versus high (11 mg·L^{-1})) in the presence and absence of effluent (control river water versus 1% effluent by volume). The low DO level was comparable with low intrasubstratum levels experienced at cold temperatures in the field (see Introduction and Discussion) and the high level was near saturation for the experimental water temperature. The experiments were run in the laboratory at the National Hydrology Research Centre using water from the South Saskatchewan River collected upstream of the City of Saskatoon. Water from this location has a chemistry (e.g., conductivity, alkalinity, pH, nitrate–nitrite, ammonium, soluble reactive phosphorus) similar to that of the Athabasca River upstream of Hinton, Alberta (Chambers and Prepas 1994; Lowell et al. 1995*a*; Culp and Podemski 1996). Full-strength treated effluent was collected from the Weldwood bleached kraft pulp mill on the Athabasca River in Hinton and stored frozen at –40°C until dilution to 1% at the beginning of the experiment. Pulp mill effluents are commonly stored deep-frozen before use in toxicity tests (Servos et al. 1996), and although the full effects of freezing are not completely understood, these experimental results were comparable with previous experiments using fresh effluent (Lowell et al. 1995*b*). The Weldwood effluent also contains Hinton municipal sewage (7.5% by volume; Culp and Podemski 1996). The 1% effluent concentration used in the experiment is typical of concentrations in the Athabasca River after the effluent plume has completely mixed across the width of the river when the river volume is at the lower levels experienced near the onset and end of the period of winter ice-cover (Culp and Podemski 1996).

The streams were circular Plexiglas chambers (diameter = 8.8 cm; Lowell et al. 1995*a*). Current was provided by small water jets driven by a vacuum chamber – pump system drawing water from control river water or 1% effluent water holding reservoirs. The water jets and pumps were adjusted so that current velocity within 1.5 cm of the bottom substratum was about 6 cm·s^{-1} so as to mimic velocities experienced by these mayflies in the field (Culp et al. 1983; Lowell et al. 1995*a*). Water returned to the holding reservoirs via a central standpipe drain in each stream, thus comprising a recirculating system. The water supplies in the holding reservoirs were renewed after the first week; freshly thawed effluent was premixed into the renewed 1% effluent water supply.

DO level in the water supplied to the low-DO (5 mg·L^{-1}) streams was controlled by passing the water through a partial vacuum chamber (Mount 1961, 1964). For each holding reservoir, water was circulated from the reservoir into an adjacent vacuum chamber utilizing the pressure differential between the reservoir and vacuum chamber. The water entered the partial vacuum chamber as a spray; the water droplets provided a large surface to volume ratio within the vacuum chamber, which facilitated rapid removal of DO from the water. DO level was controlled by varying the degree of vacuum, thereby obviating the need to mix in oxygenated water to obtain the desired DO level. The deoxygenated water was then pumped through the low-DO streams at a flow-through rate (about 1 L·min^{-1}·stream^{-1}) sufficient to ensure a uni-

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form distribution of DO within each stream as measured with a small DO probe. Control over the vacuum level and rapid flow-through rate of the distribution system also ensured a uniform DO level among all replicate streams. Water outflow from all streams was reaerated in the holding reservoirs using airstone bubblers. Water flow to the high-DO streams was provided by pumping water directly from the aerated holding reservoirs. Heat-exchange tubing was used in each reservoir to maintain equivalent temperatures between the low- and high-DO streams. This system allowed for cost-effective deoxygenation of multiple streams at more rapid flow rates than is feasible with the nitrogen-bubbling deoxygenation technique used by some other workers (e.g., Barnhart 1995), thereby facilitating long-term operation of the streams at realistic current velocities.

Larval *B. tricaudatus* were collected during the spring 10 days before the start of the experiment from Big Hill Springs Creek, Alberta, a site where several other aspects of the biology of this species have been previously studied (Scrimgeour et al. 1991; Culp and Scrimgeour 1993; Lowell et al. 1995*a*). Big Hill Springs Creek has a chemistry similar to that of the South Saskatchewan River water used during the experiment (e.g., alkalinity and pH; Walde and Davies 1984; Lowell et al. 1995*a*). Water temperature at the time of collection was about 5°C, and laboratory experiments (unpublished data) have shown that *B. tricaudatus* feed, grow, and develop at this temperature. During the 10-day acclimation period, the mayflies were held in continuously aerated 8-L containers (for both acclimation and experimental periods: temperature = 4.5°C, photoperiod = 16 h light : 8 h dark) and fed an ad libitum supply of algae (the diatom *Navicula*) cultured on 2.4 × 2.4 × 0.5 cm ceramic blocks. Identical algae-covered blocks were used to supply food to the mayflies in the streams during the experiment. At the beginning of the experiment, all mayflies in the most abundant size-class (about 6–7 mm in length; developmental stage prior to formation of black wing pads) were randomly allocated to the five replicate streams per treatment (six or seven mayflies per stream). Mayfly responses were averaged for each stream and the streams were the experimental units used for the statistical analyses. The experiment was run for 14 days.

Response variables

The key measured response variables were microhabitat positioning of animals within the streams, mayfly grazing intensity, and survival (measured daily after 8 h of light). Effects of the effluent and DO treatments on these response variables were analyzed using factorial analysis of variance (ANOVA). Proportional data (positioning behavior and survival) were arcsine square root transformed before analysis to improve data normality and variance homogeneity (Sokal and Rohlf 1995).

Microhabitat positioning of mayflies within each stream was measured by scoring the number of individuals found on each of the three categories of attachment surfaces available within each stream: (*i*) ceramic blocks, (*ii*) water jets, or (*iii*) outflow screens. The ceramic blocks were at the bottom of the streams in a region of low current velocity; velocity was lower in this region due to the drag effects imparted by the walls and floor of each stream. The water jets and outflow screens were near the upper surface of the streams in a region of high current velocity.

Grazing intensity was measured by visually scoring the areal percentage of diatom biofilm removed over the previous 24-h period from the two *Navicula*-covered food blocks placed into each stream. The accuracy of the method of scoring the areal percentage of algae removed was confirmed by computer measurement of digitized video images of the food blocks (visual score = –0.64 + 0.98 × digitized score, n = 34, SE of slope = 0.074, r = 0.92, $P < 0.001$). Although a few outlier visual scores differed by as much as 30% from the corresponding digitized score (most differences much less), the visual scores averaged within 1% of the digitized measurements and no correction factor was necessary. As algae was removed by the mayflies, the blocks were replaced at least every 3 days. In streams where grazing intensity was high, blocks were replaced more frequently to ensure that ad libitum amounts of food were always available in all treatments. It was necessary to culture the food blocks in a high-nutrient solution for a few weeks before the experiment to obtain sufficient biomass to ensure an ad libitum food supply and, given the comparatively slow algal growth rate in the streams, negligible additional growth occurred during the ≤ 3 days that they were in the streams. Furthermore, earlier trial runs indicated that any difference in algal growth on the food blocks among effluent or DO treatments over ≤ 3 days was not great enough to be detectable on a per weight basis, i.e., any algal growth differences over ≤ 3 days would have been very small relative to random interblock variations in initial algal weight and relative to the measured amount of algae removed by the mayflies. The streams were closely monitored throughout the experiment for algal growth on surfaces other than the food blocks, with particular attention paid to the surfaces (described above) where the mayflies were able to remain attached. No measurable algal growth occurred on these other surfaces during the 2-week experiment, nor was a buildup of effluent material observed over this time period, due to the dilute effluent concentration used.

Twenty-five algal-covered food blocks were set aside during the experiment for determination of the average ash-free dry mass (AFDM) of diatoms per block. This average was multiplied by percent algal film removal measured during the experiment to estimate the amount of AFDM removed per mayfly per day. The algal removal during mayfly feeding activities was due to a combination of ingestion and incidental (noningested) algal detachment and is proportional to feeding rate (Scrimgeour et al. 1991).

Results

DO level had a highly significant effect on microhabitat positioning of mayflies in the streams, with a 250–350% greater proportion of mayflies moving into regions of higher current velocity when exposed to the low-DO treatment ($P < 0.001$, ANOVA) (Fig. 1). Most mayflies exposed to the high DO level remained on the ceramic blocks at the bottom of the streams. In the low-DO treatments, however, a greater number of individuals moved up to the water jets and the outflow screens (measured each day as proportion occupying these high-velocity positions, averaged over 2 weeks, with proportions corrected for progressive losses due to mortality), the only positions within the streams where they could remain attached while exposed to higher current velocity. This upward distribution was confined to submersed regions of greater current velocity and we did not observe any individuals moving all the way up to the air–surface interface. Absence–presence of effluent and the interaction between effluent and DO did not have a significant effect on mayfly positioning in the streams ($P > 0.34$, ANOVA).

Low DO level also had a highly significant negative effect on grazing intensity ($P < 0.001$, ANOVA) (Fig. 2). Averaged over the 2-week experimental period (corrected for progressive losses due to mortality), grazing intensities when the mayflies were exposed to the low DO level were only 20% of high-DO grazing intensities, regardless of the presence of effluent. Furthermore, although not significant at the 0.05 level, the data suggest that the 1% effluent may have stimulated an increase in grazing intensity ($P = 0.08$, ANOVA). The effluent–DO interaction effect was not significant ($P = 0.36$, ANOVA).

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Fig. 1. Proportion of mayflies positioned in regions of greater current velocity within the artificial streams (proportion measured each day, averaged over 2 weeks, ±1 SE) in the absence–presence of effluent (control, 1% effluent) at two DO levels (low (5 mg·L^{-1}) and high (11 mg·L^{-1})). All remaining mayflies (not in the regions of greater current velocity) were on the ceramic blocks in the low-velocity region at the bottom of the streams. n = 5 replicate streams per treatment.

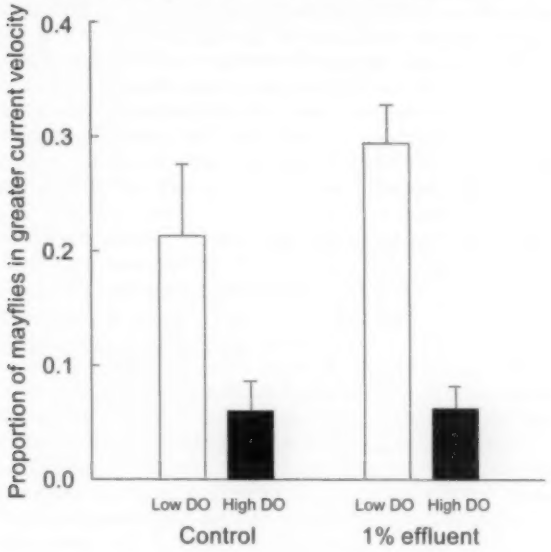
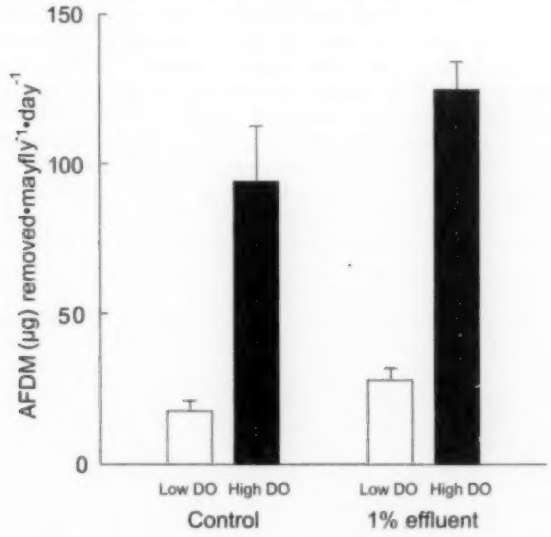
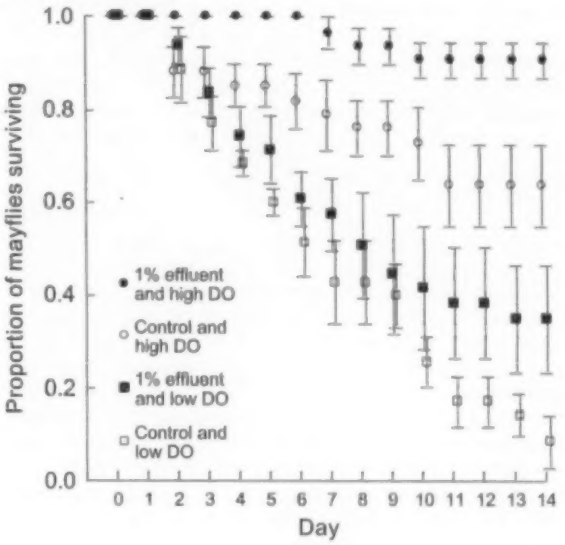


Fig. 2. Rate of food removal by mayflies (daily rate averaged over 2 weeks, ±1 SE) in the absence–presence of effluent (control, 1% effluent) at two DO levels (low (5 mg·L^{-1}) and high (11 mg·L^{-1})). n = 5 replicate streams per treatment.



By the end of the experiment, survival was reduced by 60–90% for those mayflies exposed to the low DO level (P < 0.001, ANOVA for proportion surviving in each stream on day 14) (Fig. 3). Some mortality occurred in all treatments, likely due to the late developmental stage of the mayflies; mortality typically increases in the laboratory just prior to emergence (personal observation). One individual emerged during the second week of the experiment (1% effluent – high-DO treatment). Interestingly, survival was actually enhanced for mayflies exposed to the 1% effluent (P = 0.002, ANOVA for proportion surviving in each stream on day 14). Again, the effluent–DO interaction effect was not significant (P = 0.87, ANOVA for proportion surviving in each stream on day 14).

Fig. 3. Survival of mayflies over the 14-day experimental period (±1 SE) in the absence–presence of effluent (control, 1% effluent) at two DO levels (low (5 mg·L^{-1}) and high (11 mg·L^{-1})). Points are staggered to separate error bars. n = 5 replicate streams per treatment.



Discussion

The low DO level used in this experiment clearly stressed the mayflies in several ways, leading to altered microhabitat positioning behavior, reduced grazing intensity, and decreased survival. Furthermore, these effects were observed at a DO level (5 mg·L^{-1}) comparable with those that have been measured in cold water (≤ 5°C) downriver of pulp mill and municipal effluent outfalls in the kind of intrasubstratum microhabitat used by *B. tricaudatus* in northern rivers, even when water column DO levels were near saturation (unpublished data). It is likely that DO levels in the substratum would be lower (or at least as low) at higher temperatures when substratum biochemical oxygen demand is greater (Chambers et al. 1997).

Although the experimental results address low-DO conditions that can occur in the substratum when water column DO levels are near saturation during ice-free conditions, it should be noted that the potential exists for even lower intrasubstratum DO levels when water column DO levels are significantly below saturation. This condition often exists in the

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winter when ice-cover restricts reaeration of oxygen-depleted waters downriver of pulp mill and municipal effluent outfalls (Chambers et al. 1997). Reports by Alberta Environmental Protection during recent years have typically cited minimum water column DO levels in the Athabasca River downriver of effluent outfalls of 7–8 $mg \cdot L^{-1}$, with a low of 6 $mg \cdot L^{-1}$ in 1989 (Noton and Shaw 1989; Noton and Allan 1994). Diurnal cycling of DO levels due to photosynthesis have usually led to daily fluctuations of ≤ 0.5 $mg \cdot L^{-1}$, although fluctuations as great as 2 $mg \cdot L^{-1}$ have been observed (Noton and Allan 1994). Oxygen-depleted groundwater or tributary flow into the Athabasca River may further locally depress water column DO levels to below 3 $mg \cdot L^{-1}$ (Shelast and Brayford 1995). Work with trout spawning substrata in other rivers has shown that DO levels in the substratum may be 3 $mg \cdot L^{-1}$ lower than those measured in the overlying water column, even in the absence of effluent effects on the substratum (Chapman 1986). Thus, some individuals in the Athabasca River may be exposed to DO levels significantly lower than 5 $mg \cdot L^{-1}$. On the other hand, water temperatures are typically close to 0°C during periods of ice-cover; temperatures that low may reduce metabolism (and thus, oxygen demand) sufficiently to lessen the negative impacts of low DO levels at that time of year.

Chronic stress due to insufficient oxygen delivery to the gills and reduced food intake probably explains the increased mortality caused by the low DO level during the experiment. Mortality was gradual and continuous throughout the experiment, indicating a chronic rather than acute response. Little comparable data are available on the effects of low DO levels on invertebrate survival during longer-term exposures (>1 week), although the same kind of chronic mortality response has been shown for a warmer water mayfly (*Ephemera simulans*; Nebeker 1972). These results highlight the need for conducting longer-term experiments to detect the significant impacts that can occur at DO levels that are higher than would be identified as being particularly stressful during short-term acute tests.

Survivorship in the field may be even lower due to the indirect negative effects of the altered positioning behavior caused by low DO levels. The mayflies used in our experiment moved to more elevated positions in the artificial streams following exposure to the low DO level, probably to facilitate respiration by increasing water flow past the gills, given that this species is not capable of respiratory regulation via the rhythmic beating of the gills that is exhibited by some other mayfly species (Wiley and Kohler 1980). A similar movement to more elevated positions has been described as a possible response to low-oxygen stress for several warmer water benthic invertebrates, including other *Baetis* species (Kovalak 1978). Previous studies suggest that this response would lead to increased mortality due to predation. Benthic invertebrates such as *B. tricaudatus* typically seek refuge from visual predation by fish by moving under stones, particularly during daylight hours (Culp and Scrimgeour 1993), the diel period when our data were collected (Fig. 1). Other studies have demonstrated increased predation upon benthic invertebrates when they are driven into more exposed locations by low DO levels (Rahel and Kolar 1990; Pihl et al. 1992; also see Nagell 1977; Wiley and Kohler 1980). Similarly, low DO levels can also drive small fish into more exposed areas where they are more susceptible to predators (Suthers and Gee 1986).

These studies suggest that increased exposure of benthic invertebrates to predators could occur when low DO levels in the substratum cause invertebrates to move up onto more exposed surfaces, such as the tops of rocks, woody debris, plants, or other submerged surfaces, depending on the available habitat structure. When such positioning behavior does not alleviate low-oxygen stress, it is possible that benthic invertebrates may also resort to drifting behavior, which may likewise increase exposure to predators (Wiley and Kohler 1980). The risk would likely be greatest when DO levels are low in the substratum (where the invertebrates would otherwise take refuge from fish predators) but closer to saturation in the water column, providing sufficiently high oxygen availability above the substratum to enable fish predators to actively forage.

In contrast with the negative low-DO effects, the 1% effluent treatment increased survival, at least over the 2 week course of the experiment. The effluent-associated increase in survival at the high DO level (relative to the high-DO control) was partly a consequence of unusually low survival (33%) in one replicate stream in the control – high-DO treatment. But the effluent-enhanced increase in survival at the low DO level may have been due to the possible stimulation of an increase in grazing intensity by the effluent; this may have partly offset the even greater reduction in grazing intensity caused by the low DO level. In an earlier study, we found that pulp mill effluent can stimulate increased short-term growth and development rates of *B. tricaudatus* (Lowell et al. 1995*b*). One of the mechanisms that we proposed for the growth stimulation was an effluent-induced increase in feeding rate. We also suggested other possible mechanisms for this effect, including (*i*) increased nutritive value of the periphyton and (*ii*) hormonal or other growth-stimulating effects of the compounds in pulp mill effluent. An increase in periphyton nutritive value might be due to changes in periphytic algae and (or) the deposition of effluent-derived bacteria and other organic material into the periphyton, which may supply nutrients that would otherwise be limiting. Of note, an ad libitum supply of algal food was available to the mayflies in both effluent treatments, including those in the 0% effluent treatment. This suggests an effluent-induced increase in the quality rather than the quantity of food available. Further work is required, however, to determine whether these growth-enhancing short-term effects of pulp mill effluent involve trade-offs against other longer-term fitness measures, such as fecundity.

Based on these results and data available in the literature (e.g., Lowell et al. 1995*b*, 1996, 2000; Lowell and Culp 1996; Culp et al. 2000), the negative impacts on *B. tricaudatus* (and possibly other benthic invertebrates) of pulp mill and sewage effluent in northern Canadian rivers would more likely be due to reductions in DO levels than to toxicant effects, at least over the short term. The measured cumulative effects appear to be largely additive (Suter 1993), and the data further suggest that the negative impacts of low DO levels will outweigh the nutrient enhancement effects of these effluents in some areas. Thus, future monitoring efforts should pay particular attention to DO levels. Intrasubstratum DO levels downriver of effluent outfalls in some of these rivers may al-

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ready be low enough to have had deleterious effects on some benthic invertebrates and, where possible, further reductions in DO levels should be avoided.

Assessments of potential present and future impacts would benefit from followup experiments designed to address the following points. Impacts may be modified by differences in the developmental stage of larvae and the length of exposure, including acclimation time to low DO levels. Different species, as well as individuals collected from different riverine environments, are also likely to vary in their tolerance to toxicants and low DO levels. Higher contaminant concentrations experienced closer to effluent outfalls, and during periods when the effluent to river discharge ratio is particularly high, may also modify impacts. In addition, effluent properties can change temporally both within and among mills. Finally, studies of the combined effects of effluent and low intrasubstratum DO levels on fish–invertebrate predator–prey interactions at moderate to low temperatures would further facilitate predictions of future impacts of pulp mill and sewage effluents in northern rivers.

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References

Barnhart, M.C. 1995. An improved gas-stripping column for deoxygenating water. J. North Am. Benthol. Soc. **14**: 347–350.

Birtwell, I.K. 1989. Comments on the sensitivity of salmonids to reduced levels of dissolved oxygen and to pulp mill pollution in Neroutsos Inlet, British Columbia. Can. Tech. Rep. Fish. Aquat. Sci. No. 1695.

Chambers, P.A. 1996. Nutrient enrichment in the Peace, Athabasca and Slave rivers: assessment of present conditions and future trends. Synth. Rep. No. 4. Northern River Basins Study, Edmonton, Alta.

Chambers, P.A., and Prepas, E.E. 1994. Nutrient dynamics in riverbeds: the impact of sewage effluent and aquatic macrophytes. Water Res. **28**: 453–464.

Chambers, P.A., Scrimgeour, G.J., and Pietroniro, A. 1997. Winter oxygen conditions in ice-covered rivers: the impact of pulp mill and municipal effluents. Can. J. Fish. Aquat. Sci. **54**: 2796–2806.

Chapman, G. 1986. Ambient water quality criteria for dissolved oxygen. EPA 440/5-86-003. U.S. Environmental Protection Agency, Washington, D.C.

Culp, J.M., and Podemski, C.L. 1996. Impacts of contaminants and nutrients in bleached kraft mill effluent on benthic insect and periphyton communities: assessments using artificial streams, Athabasca River, 1993 and 1994. Project Rep. No. 92. Northern River Basins Study, Edmonton, Alta.

Culp, J.M., and Scrimgeour, G.J. 1993. Size-dependent diel foraging periodicity of a mayfly grazer in streams with and without fish. Oikos, **68**: 242–250.

Culp, J.M., Walde, S.J., and Davies, R.W. 1983. Relative importance of substrate particle size and detritus to stream benthic macroinvertebrate microdistribution. Can. J. Fish. Aquat. Sci. **40**: 1568–1574.

Culp, J.M., Lowell, R.B., and Cash, K.J. 2000. Integrating *in situ* community experiments with field studies to generate weight-of-evidence risk assessments for large rivers. Environ. Toxicol. Chem. **19**. In press.

Davis, J.C. 1975. Minimal dissolved oxygen requirements of aquatic life with emphasis on Canadian species: a review. J. Fish. Res. Board Can. **32**: 2295–2332.

Graves, W.C., Burton, D.T., Richardson, L.B., and Margrey, S.L. 1981. The interaction of treated bleached kraft mill effluent and dissolved oxygen concentration on the survival of the developmental stages of the sheepshead minnow (*Cyprinodon variegatus*). Water Res. **15**: 1005–1011.

Hicks, D.B., and DeWitt, J.W. 1971. Effects of dissolved oxygen on kraft pulp mill effluent toxicity. Water Res. **5**: 693–701.

Kovalak, W.P. 1978. Diel changes in stream benthos density on stones and artificial substrates. Hydrobiologia, **58**: 7–16.

Kruzynski, G.M. 1979. Some effects of dehydroabietic acid (DHA) on hydromineral balance and other physiological parameters in juvenile sockeye salmon *Oncorhynchus nerka*. Ph.D. thesis, University of British Columbia, Vancouver, B.C.

Lowell, R.B., and Culp, J.M. 1996. Sampling strategies for environmental effects monitoring: implications for sampling frequency based on a long-term benthic program in the Thompson River, British Columbia. DOE FRAP 1996-07. Fraser River Action Plan, Environment Canada, Vancouver, B.C.

Lowell, R.B., Culp, J.M., and Wrona, F.J. 1995*a*. Toxicity testing with artificial streams: effects of differences in current velocity. Environ. Toxicol. Chem. **14**: 1209–1217.

Lowell, R.B., Culp, J.M., and Wrona, F.J. 1995*b*. Stimulation of increased short-term growth and development of mayflies by pulp mill effluent. Environ. Toxicol. Chem. **14**: 1529–1541.

Lowell, R.B., Culp, J.M., Wrona, F.J, and Bothwell, M.L. 1996. Effects of pulp mill effluent on benthic freshwater invertebrates: food availability and stimulation of increased growth and development. *In* Environmental fate and effects of pulp and paper mill effluents. *Edited by* M.R. Servos, K.R. Munkittrick, J.H. Carey, and G.J. Van Der Kraak. St. Lucie Press, Delray Beach, Fla. pp. 525–532.

Lowell, R.B., Culp, J.M., and Dubé, M.G. 2000. A weight-of-evidence approach for northern river risk assessment: integrating the effects of multiple stressors. Environ. Toxicol. Chem. **19**. In press.

Mount, D.I. 1961. Development of a system for controlling dissolved-oxygen content of water. Trans. Am. Fish. Soc. **90**: 323–327.

Mount, D.I. 1964. Additional information on a system for controlling the dissolved oxygen content of water. Trans. Am. Fish. Soc. **93**: 100–103.

Nagell, B. 1977. Phototactic and thermotactic responses facilitating survival of *Cloeon dipterum* (Ephemeroptera) larvae under winter anoxia. Oikos, **29**: 342–347.

Nebeker, A.V. 1972. Effect of low oxygen concentration on survival and emergence of aquatic insects. Trans. Am. Fish. Soc. **101**: 675–679.

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Noton, L.R., and Allan, D. 1994. Oxygen conditions in the Athabasca River system, with emphasis on winters 1990–1993. Alberta Environmental Protection, Technical Services and Monitoring Division, Edmonton, Alta.

Noton, L.R., and Shaw, R.D. 1989. Winter water quality in the Athabasca River system. Alberta Environment, Environmental Assessment Division, Environmental Quality Monitoring Branch, Edmonton, Alta.

Pihl, L., Baden, S.P., Diaz, R.J., and Schaffner, L.C. 1992. Hypoxia-induced structural changes in the diet of bottom-feeding fish and Crustacea. Mar. Biol. **112**: 349–361.

Rahel, F.J., and Kolar, C.S. 1990. Trade-offs in the response of mayflies to low oxygen and fish predation. Oecologia, **84**: 39–44.

Schallock, E.W., and Lotspeich, F.B. 1974. Low winter dissolved oxygen in some Alaskan rivers. EPA-660/3-74-008. U.S. Environmental Protection Agency, Corvallis, Oreg.

Scrimgeour, G.J., Culp, J.M., Bothwell, M.L., Wrona, F.J., and McKee, M.H. 1991. Mechanisms of algal patch depletion: importance of consumptive and non-consumptive losses in mayfly–diatom systems. Oecologia, **85**: 343–348.

Servos, M.R., Munkittrick, K.R., Carey, J.H., and Van Der Kraak, G.J. (*Editors*). 1996. Environmental fate and effects of pulp and paper mill effluents. St. Lucie Press, Delray Beach, Fla.

Shelast, B.M., and Brayford, K.T. 1995. Winter water quality survey on the Athabasca River, February 1995. Project Rep. 09-805-00. Alberta Newsprint Company, Whitecourt, Alta.

Sokal, R.R., and Rohlf, F.J. 1995. Biometry. W.H. Freeman, New York.

Suter, G.W. 1993. Ecological risk assessment. Lewis Publishers, Chelsea, Mich.

Suthers, I.M., and Gee, J.H. 1986. Role of hypoxia in limiting diel spring and summer distribution of juvenile yellow perch (*Perca flavescens*) in a prairie marsh. Can. J. Fish. Aquat. Sci. **43**: 1562–1570.

Walde, S.J., and Davies, R.W. 1984. Invertebrate predation and lotic prey communities: evaluation of in situ enclosure/exclosure experiments. Ecology, **65**: 1206–1213.

Wiley, M.J., and Kohler, S.L. 1980. Positioning changes of mayfly nymphs due to behavioral regulation of oxygen consumption. Can. J. Zool. **58**: 618–622.

Winter, A., Ciborowski, J.J.H., and Reynoldson, T.B. 1996. Effects of chronic hypoxia and reduced temperature on survival and growth of burrowing mayflies, *Hexagenia limbata* (Ephemeroptera: Ephemeridae). Can. J. Fish. Aquat. Sci. **53**: 1565–1571.

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Dimethylsulfoxide (DMSO) and related sulfur compounds in the Saguenay Fjord, Quebec

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Abstract: The biogeochemical cycling of dimethylated sulfur compounds was investigated in the Saguenay Fjord during June 1996, just prior to the flooding in the catchment area. Dimethylated sulfur compounds, namely dimethylsulfide (DMS), dimethylsulfoniopropionate (DMSP), and dimethylsulfoxide (DMSO), were determined at six sites. In comparison with other coastal environments, including the Gulf of St. Lawrence, DMS (0–1.8 nmol·L^{-1}, average <0.2 nmol·L^{-1}) and DMSP (0–23 nmol·L^{-1}, average <0.8 nmol·L^{-1}) concentrations were relatively low because the brackish waters were poor in algal abundance and dominated by species not noted as major DMS and DMSP producers. Similarly, dissolved DMSO levels (<0.016 nmol·L^{-1}) were low due to the limited availability of its DMS precursor and the shallow photic zone. Particulate DMSO was measured (0–110 nmol·L^{-1}, average ~8 nmol·L^{-1}) and occasionally exceeded particulate DMSP concentrations (0.016–140 nmol·L^{-1}, average ~3.5 nmol·L^{-1}). Given the low dissolved DMSO concentrations, the presence of particulate DMSO provides evidence for its direct biosynthesis by phytoplankton.

Résumé : Le cycle biogéochimique des composés de soufre diméthylés a été étudié dans le fjord du Saguenay en juin 1996, quelques semaines avant la crue éclair survenue dans la région du Saguenay – Lac-Saint-Jean. Les concentrations aqueuses des différents composés du soufre, le sulfure de diméthyle (DMS), le diméthylsulfoniopropionate (DMSP) et le sulfoxyde de diméthyle (DMSO), ont été mesurées à six stations le long du fjord. En comparaison avec d'autres milieux côtiers, incluant le golfe du Saint-Laurent, les concentrations en DMS (0–1,8 nmol·L^{-1}, moyenne <0,2 nmol·L^{-1}) et en DMSP (0–23 nmol·L^{-1}, moyenne <0,8 nmol·L^{-1}) sont relativement faibles car les eaux saumâtres du Fjord sont pauvres en cellules algales et dominées par des taxons non reconnus pour être de grands producteurs de DMS et de DMSP. De façon similaire, de faibles teneurs en DMSO dissous (<0,016 nmol·L^{-1}) ont été rencontrées dans toute la colonne d'eau en raison d'une disponibilité limitée de son précurseur, le DMS, et d'une couche photique peu profonde. Par contre, les concentrations en DMSO particulaire (0–110 nmol·L^{-1} moyenne ~8 nmol·L^{-1}) ont excédé parfois celles en DMSP particulaire (0,016–140 nmol·L^{-1}, moyenne ~3,5 nmol·L^{-1}). Étant donné les faibles concentrations en DMSO dissous, la présence de DMSO particulaire supporte l'hypothèse d'une biosynthèse directe du DMSO par le phytoplancton.

Introduction

The occurrence of dimethylsulfide (DMS) in the ocean is interrelated to a number of compounds, in particular to dimethylsulfoniopropionate (DMSP) and dimethylsulfoxide (DMSO). Together, these sulfur compounds comprise a complicated biogeochemical system involving living and detrital particulate material, soluble chemical species, and dissolved gases. In the marine biogenic sulfur cycle, attention has focussed on DMS because it can influence climate in several ways: the hydrological cycle, precipitation acidity in remote regions, and the global radiation balance. The transfer of DMS to the atmosphere may involve as little as 10% of the available DMS (Malin et al. 1992; Kieber et al. 1996), indicative that other processes influence the biogeochemical cycling of these sulfur compounds in surface marine waters. The origin of DMS is DMSP, which behaves as an osmoregulator and cryoprotectant and is produced by marine micro- and macro-algae (see review by Liss et al. 1997). Intracellular DMSP production is strongly species dependent (Turner et al. 1988). Cell senescence results in the release of DMSP into the aqueous phase. This release can be augmented by zooplankton grazing and viral infection of algal cells (see review by Liss et al. 1997).

Most studies have focussed on DMSP as the precursor for DMS, but the partitioning of sulfur between DMS and DMSO is also important (Malin et al. 1992). Circumstantial evidence indicates that DMSO derives ultimately from a biogenic origin, but the formation pathways remain somewhat conjectural. Conventionally, it is believed that DMSO results from the photooxidation of DMS (Kieber et al. 1996). A study of DMSO in coastal waters of New Zealand (Lee and de Mora 1996) did show that DMSO concentrations exhibited a diurnal variation, demonstrating daytime production. Also, laboratory studies have shown that bacteria can facilitate interconversion of DMS and DMSO. While

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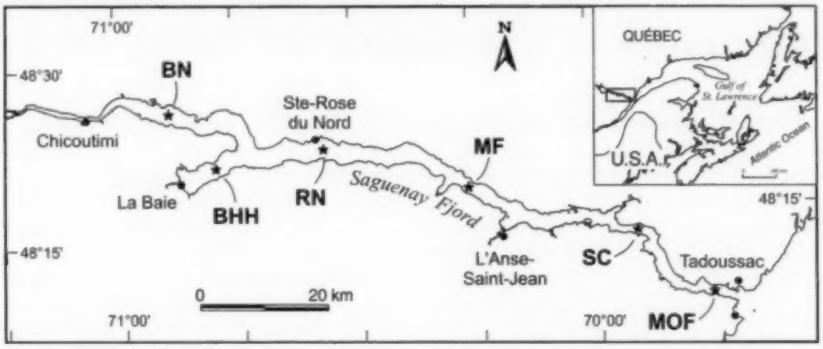
Fig. 1. Map of Saguenay Fjord showing the six sampling sites.



this has yet to be demonstrated in oxygenated seawater, it has been argued that this may be important (Liss et al. 1997). Noting that dissolved DMSO concentrations may be an order of magnitude greater than those of DMS (Hatton et al. 1996; Lee and de Mora 1996), small perturbations in the DMSO content could stimulate large variations in dissolved DMS concentrations. As a further uncertainty in the biogeochemical cycling of DMS-related compounds, DMSO is widely found in natural foodstuffs (Pearson et al. 1981) and could reasonably be expected to be directly biosynthesized by organisms.

There have been relatively few studies of DMS and DMSP in Canadian waters, and to date, none have considered DMSO biogeochemistry. In the northern Gulf of St. Lawrence, Levasseur et al. (1994) (also see the Ackman and Sipos references cited therein) examined the relationship between phytoplanktonic DMSP and blackberry feed malodours in Atlantic cod (*Gadus morhua*). The distributions of DMSP and DMS, together with the importance of zooplankton grazing, have been presented for the central Gulf of St. Lawrence (Cantin et al. 1996) and the ensuing DMS flux to the atmosphere has been modelled (Levasseur et al. 1997). The primary objective of this study was to examine the distribution and short-term variability of DMS, its precursor DMSP, and its oxidation product DMSO in the Saguenay Fjord. Secondary goals for the project were to extend recent laboratory experiments looking at particulate-phase DMSO in phytoplankton and cyanobacteria cultures to natural phytoplanktonic communities and examine its potential impact on the biogeochemical cycling of sulfur.

Materials and methods

Site description

The Saguenay Fjord is a classic fjord located on the north shore of the St. Lawrence Estuary in central Québec. Features of the Saguenay Fjord, including geological and biological aspects, have been reviewed relatively recently (Schafer et al. 1990). The fjord displays archetypal characteristics, being a long (170 km), narrow (1–6 km) channel contained within a U-shaped valley. The fjord is defined at the mouth by a shallow sill (about 20 m depth) at Tadoussac but is further subdivided by a deeper sill (80 m) 18 km upstream. These sills thereby define two basins, which reach depths of 250 and 275 m for the eastern (or outer) and western (inner) basins, respectively. In some studies, the western basin is considered as being further subdivided due to a deepwater sill (120 m) about 40 km upstream from Tadoussac (Drainville 1968).

Typical estuarine circulation is maintained by the presence of a strong thermohalocline. The fjord is strongly stratified, having a relatively thin buoyant (warm, 16–18°C in the summer, and fresh, salinity <5 psu) layer overlying a much deeper saline water mass (Drainville 1968). The freshet occurs in late spring or early summer. Large intrusions of cold, well-oxygenated water that affect the basins down to 150 m occur in the winter. Interpretation of particle size distributions has indicated that the two-layer system evolves into one of three layers during the summer months (Chanut and Poulet 1979), with relatively high sediment burdens being present in the surface and deepest layers. The photic zone tends to be limited to the upper 10 m, and hence, primary productivity is much lower than in adjacent waters of the St. Lawrence. Nevertheless, deeper waters (down to 100 m) exhibit relatively elevated levels of chlorophyll during the summer (Schafer et al. 1990).

Exchange of water between the outer two basins of the Saguenay Fjord and the St. Lawrence Estuary is thought to occur more frequently than in many other fjords, possibly due to the existence of large-amplitude internal waves in the St. Lawrence Estuary (De Ladurantaye et al. 1984). These authors found that the composition of zooplankton and variations in abundance near the mouth of the fjord were strongly controlled by processes taking place in the estuary and concluded that the establishment of a local population was difficult. Conversely, at upstream sites, where the influence of advective processes was lower, endogenous populations could develop and thrive under ambient conditions. Adding to the complexity are considerable current variations that have been observed in the fjord. Within the first basin, Therriault et al. (1984) observed currents up to 2 $m \cdot s^{-1}$ in the surface layer and 1.4 $m \cdot s^{-1}$ near the bottom, and Gratton et al. (1993) found that surface currents could vary from 0.4 to 1.4 $m \cdot s^{-1}$ within a tidal cycle.

Sample collection

Water samples were collected at six stations in the Saguenay Fjord (Fig. 1; Table 1) during a 12-day period between June 3 and 14, 1996. All sampling was carried out adrift, and due to the strong currents in the fjord, the ship was repositioned at the start of each sample collection. Two types of stations were occupied during the cruise. The stations Baie des Ha! Ha! (BHH), midfjord (MF), and mouth of fjord (MOF) were occupied for a period of 48 h. Commencing at midnight, four depth profiles with a periodicity of 12 h were obtained at these three stations. In addition to these profiles, two depths (2 and 15 m) were sampled every 2 h to investigate short-term temporal variations. At the remaining stations, Le bras Nord (BN), Ste-Rose du Nord (RN), and Sacré-Coeur (SC), two depth profiles were collected with a sampling periodicity of 12 h.

With the exception of the shallow westernmost stations BN and BHH, samples were collected from depths of 2, 5, 10, 15, 25, 50,

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Table 1. Sampling dates and positions occupied in the Saguenay Fjord in June 1996 together with the average (standard deviation) physical characteristics.

	BN	BHH	RN	MF	SC	MOF
Date	June 11	June 9–10	June 7	June 5–6	June 3	June 13–14
Latitude (N)	48°25.51′	48°20.96′	48°22.21′	48°17.86′	48°13.97′	48°08.38′
Longitude (W)	70°52.42′	70°47.88′	70°34.95′	70°15.81′	69°55.36′	69°46.07′
Water depth (m)	51	156	264	279	189	248
Mixed layer depth (m)	3.25 (0.35)	5.75 (0.96)	7.50 (0.71)	7.50 (1.00)	9.75 (0.35)	4.25 (0.96)
Euphotic layer depth (m)	4.1 (1.0)	3.4 (0.5)	3.7 (0.4)	4.0 (0.4)	3.8 (0.9)	5.8 (0.9)
Salinity stratification index	1.0 (0.1)	0.86 (0.02)	0.93 (0.004)	0.90 (0.03)	0.86 (0.01)	0.51 (0.05)

Note: The euphotic layer depth is defined as the depth receiving 1% of the surface irradiance. The depth of the mixed layer is defined as the depth where the gradient in σ_t was maximum. The salinity stratification index is defined as the salinity in the deep layer minus the salinity at the surface divided by the average salinity (Dyer 1973).

100, and 150 m using a General Oceanics rosette equipped with 5-L Niskin bottles. Vertical profiles of temperature and salinity were determined at each station using a Guildline digital CTD attached to the sampling rosette. In the case of stations with depths greater than 150 m, additional water samples were collected with 12-L Niskin bottles individually mounted on a hydrographic wire. Water samples were taken at each depth for the determination of several biological and chemical variables. An initial subsample was cautiously taken immediately after sampling and set aside for the determination of DMS and dissolved and particulate DMSP and DMSO ($DMSP_d$, $DMSP_p$, $DMSO_d$, $DMSO_p$). A second subsample was used to measure the chlorophyll *a* (Chl *a*) and phaeopigment concentrations as well as the concentrations of inorganic nutrients. A third subsample was taken for the determination of particle size distributions and either used immediately for direct counting or preserved by adding acidic Lugol's solution (Parsons et al. 1984) for later (within 1 week) measurements in a shore-based laboratory. Finally, subsamples were preserved in acidic Lugol's solution (Throndsen 1978), gluteraldehyde–paraformaldehyde solution (Tsuji and Yanagita 1981), and alkaline formaldehyde solution for subsequent enumeration and identification of phytoplankton.

Biological and nutrient measurements

Subsamples for pigment analyses were filtered on Whatman GF/F glassfibre filters and on Poretics polycarbonate 5-μm membranes. Concentrations of pigments (Chl *a* and phaeopigments) were determined on a R010 Turner Designs fluorometer after extraction for 24 h in 90% acetone at 5°C without grinding (Parsons et al. 1984). Particle size distributions were determined on a Coulter Counter model TA II. Particle size analysis was performed in triplicate in the size range 1.0–180 μm by using two sensors with aperture diameter of 70 or 280 μm. After sedimentation of the fixed water samples in settling chambers (Utermöhl 1958), the chambers were examined under a Zeiss Axiovert 100 inverted microscope. The Lugol-preserved samples were counted at 400× magnification under Normarski differential interference contrast (DIC) optics, while glutaraldehyde samples and smaller species were checked at 1000× magnification using epifluoresence microscopy in addition to DIC optics (Lovejoy et al. 1993). Seawater subsamples for nutrient analysis were filtered through Whatman GF/F filters and the filtrate frozen at –20°C for later analysis in a shore-based laboratory. Analyses for the nutrients $NO_3^- + NO_2^-$, $Si(OH)_4^-$, and PO_4^{3-} were carried out using a Perstorp Analytical Enviro Flow 3500 (Alpkem, I.O. Analytical).

Sulfur determinations

All sulfur determinations were carried out using Varian Star 3400CX gas chromatographs equipped with either a flame photometric detector (GC-FPD) or a pulsed flame photometric detector (GC-PFPD). Both instruments were equipped with flow controllers to allow introduction of SF_6 to improve flame sensitivity (de Mora et al. 1993). During the cruise, DMS concentrations were determined immediately after collection of water samples using a purge-and-trap preconcentration technique (de Mora et al. 1990). Essentially, the chromatographic analysis of DMS was carried out using a Carbopack B-packed teflon column (Supelco Inc.), with temperature programming being used to facilitate baseline separation of DMS and CS_2. Calibration of the GC for DMS was carried out using permeation tubes (Shooter et al. 1992). A peristaltic pump was used to pull DMS from the permeation tube through the GC's six-port rotary valve and into the sample loop. The sample valve was then opened and the contents of the sample loop flushed into the GC. The detection limit for DMS was 5 $pmol \cdot L^{-1}$ (standard deviation 26%) when a 100-mL (maximum volume) sample was sparged.

All DMSP samples were collected and stored for later analysis in a shore-based laboratory. For $DMSP_p$ analyses, water samples were filtered through Whatman GF/F filters, with between 0.5 and 2 L of sample being filtered depending on the quantity of particles present. A 48-mL aliquot of filtered water sample was stored in 25-mL Wheaton serum bottles (with aluminium seals and butyl stoppers) for subsequent $DMSP_d$ analyses. Sodium hydroxide was added to the DMSP samples to convert DMSP to DMS and the samples were frozen. DMSP was analysed as DMS following procedures previously outlined. The DMSP detection limit was 8 $pmol \cdot L^{-1}$ as DMS equivalents with a standard deviation of 18%.

Samples for $DMSO_d$ analyses were filtered (Whatman GF/F) immediately upon collection. A 2-mL aliquot of chloroform was added as a preservative to a 48-mL aliquot of the filtrate. Samples were stored in the dark (50-mL Wheaton serum bottles with aluminium seals and butyl stoppers) until subsequent analysis. Between 250 and 1000 mL of seawater was filtered, and immediately thereafter the filters were wrapped in aluminium foil, frozen in liquid nitrogen, and stored at –22°C for later $DMSO_p$ measurements. At that stage, about 3 mL of 95% ethanol was added to a filter, which was then ground twice using a teflon grinder under dark and cool conditions. To ensure complete extraction, the solutions were placed in an ultrasonic bath for 15 min and stored in the dark at room temperature for 24 h. Finally, the extracts were centrifuged for 15 min at 3700 rpm, decanted, and made up to a volume of 5 mL with ethanol. The aqueous solutions ($DMSO_d$) and ethanol-based extracts ($DMSO_p$) were analysed using a direct injection technique with sample volumes in the range of 5–30 μL (de Mora et al. 1993). Unlike reduction techniques for DMSO (vide infra), the direct method is not subject to any interferences from DMSP or other intracellular sulfur compounds containing the DMS moiety. Hence, the method does not require any such interferent in the sample to be either removed before analysis or corrected for post-analysis. Thus, the DMSO measured is the actual DMSO present in the filtered phytoplankton sample.

Chromatography for DMSO was carried out using temperature

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Table 2. Mean and range of concentrations (nmol·L^{-1}) for DMS, $DMSP_d$, $DMSP_p$, $DMSO_d$, and $DMSO_p$ observed at each station.

	BN	BHH	RN	MF	SC	MOF
DMS						
Mean	0.14	0.11	0.028	na	0.012	0.012
Range	0–0.68	0–1.8	0–0.15	na	0–0.06	0–0.24
$DMSP_d$						
Mean	nd	0.65	0.28	na	na	0.30
Range	nd	0–23	0–1.03	na	na	0–10
$DMSP_p$						
Mean	2.9	1.07	0.48	0.70	0.68	15
Range	0.084–11	0.016–28	0.049–1.8	0.024–10	0.032–4.0	0.03–140
$DMSO_d$						
Mean	nd	nd	nd	nd	na	nd
Range	nd	nd	nd	nd	na	nd
$DMSO_p$						
Mean	na	10	na	7.8	na	7.2
Range	na	0–110	na	0–30	na	0–26

Note: na, not analysed; nd, not detected.

programming (1 min at 110°C, 20°C·min^{-1}, 2 min at 150°C) on a 2-m teflon column packed with 15% free fatty acid phase (FFAP) on 40- to 60-mesh Chromosorb T (Supelco Inc.). The detection limit for DMSO was 2.5 nmol·L^{-1}. Some $DMSO_d$ samples were reanalysed using a reduction technique (Simó et al. 1997). After the samples were stripped of DMS and DMSP, sodium borohydride was added to reduce DMSO to DMS. The samples were then analysed for DMS as outlined above. With this method, the detection limit for DMSO was 16 pmol·L^{-1}.

Statistical methods

Data were checked for their Gaussian distribution and were transformed logarithmically prior to analysis if this was not the case. This transformation also ensured a linear relationship between the variables of interest. Pearson correlation coefficients and trends in data were computed using the SYSTAT statistical package (Wilkinson et al. 1992). Nonparametric correlation (Spearman and Kendall) coefficients were also calculated to validate significant values.

Results

Ranges and mean values for the dimethylated sulfur compounds DMS, DMSP, and DMSO are presented in Table 2 for each station in the Saguenay Fjord. Representative average profiles, from stations BHH and MOF, are illustrated in Fig. 2. Details regarding the taxonomic composition of the phytoplankton and microzooplankton communities observed at stations BHH and MOF are given in Table 3. The low levels of dimethylated sulfur measured in the Saguenay Fjord (Table 2) derived from a phytoplankton community that consisted principally of brackish water flagellates and was generally devoid of known DMSP-producing species such as *Phaeocystis* sp. and *Emiliania huxleyi*. The total abundance of plankton cells and flagellates at 2 m were two and five times higher, respectively, at station MOF than at station BHH (Table 3). Both stations were dominated by autotrophic (pigmented) flagellates, the large (35–60 μm) Euglenophyceae *Eutreptiella marina* and the small (7–10 μm) cryptophyte *Pelagioselmis* spp. The main heterotrophic flagellates belonged to the genus *Bodo*. The second most abundant group was diatoms. The pennate *Asterionella formosa* and the centric *Aulacoseira granulata* were the most abundant diatoms at station BHH, while the centric *Skeletonema costatum* was the dominant species of this algal group at station MOF. At both stations, the most abundant dinoflagellates and oligotrichous ciliates were *Gymnodinium* spp. and *Strombidium* spp., respectively. There were no significant differences in the abundances of diatoms, dinoflagellates, ciliates, and other protozoans between the two stations.

While $DMSP_p$ levels ranged between 0.016 and 138 nmol·L^{-1}, concentrations were generally very low. Over 70% of the samples analysed contained less than 1 nmol·L^{-1} $DMSP_p$, and of those samples containing more than 1 nmol·L^{-1} $DMSP_p$, 53% occurred at station MOF. These concentrations are comparable with the lowest levels in estuaries along the east coast of the United States (Iverson et al. 1989). The $DMSP_p$ concentrations within the Saguenay Fjord were also generally less than those found for the northern Gulf of St. Lawrence (<0.3–59 nmol·L^{-1}, Levasseur et al. 1994) and for the central Gulf of St. Lawrence (6–177 nmol·L^{-1}, Cantin et al. 1996). In general, the values for the Saguenay Fjord were lower than values determined in coastal waters around the British Isles (14–288 nmol·L^{-1}, Turner et al. 1988) and in a Norwegian fjord (up to 170 nmol·L^{-1}, Bratbak et al. 1995).

Although the highest $DMSP_d$ concentrations (up to 23.5 nmol·L^{-1}) observed in the Saguenay Fjord are comparable with those of the Gulf of St. Lawrence (1.7–23.1 nmol·L^{-1}), most samples contained less $DMSP_d$ than the lowest values determined in the central Gulf of St. Lawrence (Cantin et al. 1996). These levels of $DMSP_d$ are also less than those measured in other coastal environments, such as for coastal waters around the British Isles (5.6–198 nmol·L^{-1}, Turner et al. 1988). Similarly, DMS concentrations were very low (not detected to 1.8 nmol·L^{-1}). These values are about four times lower than those observed for the Gulf of St. Lawrence (<0.9–9 nmol·L^{-1}, Cantin et al. 1996). DMS levels were also between three and 20 times lower than DMS levels observed in studies carried out in other regions (0.4–34.3 nmol·L^{-1}, Turner et al. 1988; 1–18 nmol·L^{-1},

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Table 3. Average (standard error) abundance of the various planktonic groups (at 2 m) for stations BHH and MOF in June 1996.

	BHH		MOF	
Taxonomic group	10^3 cells·L^{-1}	% total	10^3 cells·L^{-1}	% total
Pennate diatoms	59.7 (34.9)	22.1	53.3 (10.5)	10.6
Centric diatoms	17.9 (13.6)	6.6	65.0 (20.2)	12.9
Autotrophic dinoflagellates	2.9 (2.9)	1.1	3.1 (1.2)	0.6
Heterotrophic dinoflagellates	10.7 (9.2)	4.0	2.1 (0.9)	0.4
Autotrophic flagellates	80.3 (68.6)	29.7	229.0 (35)	45.6
Heterotrophic flagellates	70.5 (18.0)	26.1	128.7 (33.8)	25.6
Oligotrichous ciliates	3.5 (3.5)	1.3	2.4 (1.1)	0.5
Other protozoans	25.1 (4.1)	9.3	19.0 (4.5)	3.8
Total	270.6 (3.6)		502.7 (52.8)	

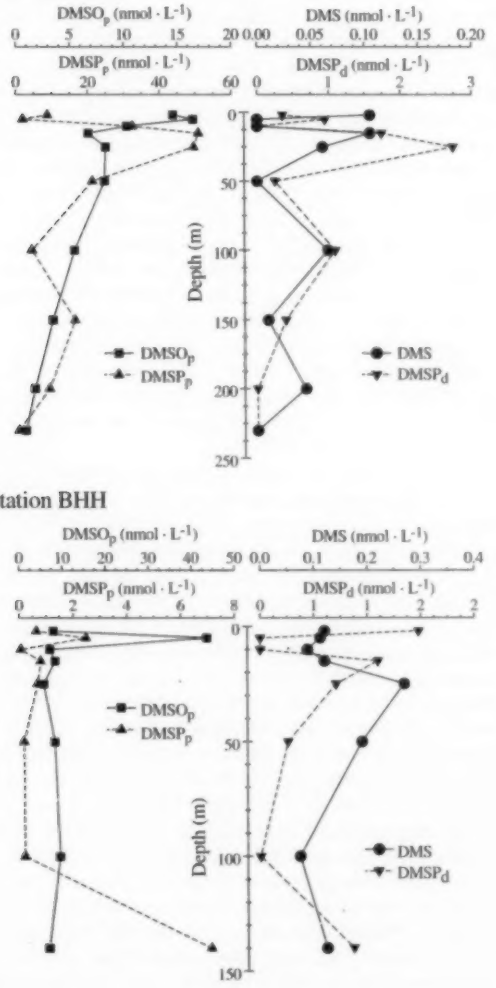

Fig. 2. Vertical profiles for DMS, DMSP$_d$, DMSP$_p$, and DMSO$_p$ at (*a*) station MOF and (*b*) station BHH.

Iverson et al. 1989; 0.15–27 nmol·L^{-1}, Turner et al. 1995; 0–19.3 nmol·L^{-1}, Simó et al. 1997). Total DMSP$_d$ + DMS values are also generally lower than those found for the northeastern Gulf of St. Lawrence and Strait of Belle Isle (not detected to 39 nmol·L^{-1}, Levasseur et al. 1994), and during an *Emiliania huxleyi* bloom in a Norwegian fjord, concentrations of total DMSP$_d$ + DMS up to 50 nmol·L^{-1} were measured in the fjord and up to 80 nmol·L^{-1} in mesocosm enclosures (Bratbak et al. 1995).

Discussion

A considerable amount of variability was observed during experiments to examine temporal variability (data not shown). It was not uncommon to measure extreme values within the range of values observed at a given station within a 4-h time period. As a result of this short-term variability, no diurnal trends could be detected for any of the sulfur compounds under investigation. Similarly, many of the interrelationships that are observed between these sulfur compounds in other marine environments were not generally found in the Saguenay Fjord. Some exceptions to this were seen at station MOF. Significant correlations were observed between DMS and DMSP$_p$ ($r = 0.559$, $p \leq 0.05$) and between DMS and the number of particles in the 9- to 30-μm range ($r = 0.599$, $p \leq 0.01$). DMSP$_p$ was found to be correlated with the number of particles in the 9.0- to 30.0- and 30.0- to 90.0-μm size-classes ($r = 0.596$, $p \leq 0.01$ and $r = 0.664$, $p \leq 0.01$, respectively). Typically, no correlation was observed between DMSP$_p$ and Chl *a*. However, the general lack of a correlation between DMSP$_p$ and Chl *a* is not unexpected given that the ability of phytoplankton species to produce DMSP can vary considerably (Turner et al. 1988). For the Saguenay Fjord, the poor correlation between DMSP$_p$ and Chl *a* would be further exacerbated because the Chl *a* maximum occurs in the upper part of the euphotic zone within the low-salinity surface layer, whereas the DMSP$_p$ maximum is generally observed in the more saline layer at the bottom of the euphotic zone.

Although a slight downward trend in DMSP$_p$ concentrations was observed in going from the head to the mouth of the fjord, the differences were not significant. However, the DMSP$_p$ levels found at station MOF at the mouth of the

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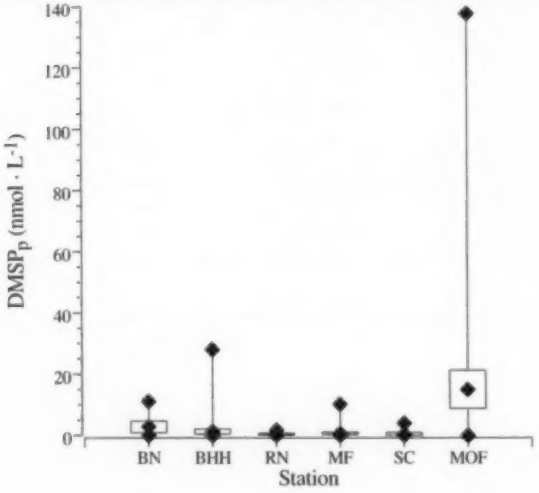
Fig. 3. Box and whisker diagram of $DMSP_p$ concentrations (mean, range, 95% CI) along the Saguenay Fjord.



fjord were significantly higher than those for the rest of the fjord (Fig. 3). The same trend was observed when calculations were made for data from the entire water column and when data were divided into groups corresponding to the surface layer and the lower, more saline layer. Furthermore, a significant correlation was observed between $DMSP_p$ and autotrophic dinoflagellates ($r = 0.700$, $p \leq 0.05$) at the mouth of the fjord. Conversely, for DMS and $DMSP_d$, no significant differences were observed among any of the stations occupied including station MOF.

Degradation of these sulfur compounds may have also been enhanced in the fjord environment. Average concentrations of $DMSP_d$ were generally greater than those of DMS (Table 2; Fig. 2). The ratio of $DMSP_d$ to DMS did show some large variations as a result of the temporal variability caused by the current flow. Typically, $DMSP_d$ levels were 5–50 times greater than those of DMS. In comparison with other studies, the ratios of $DMSP_d$ to DMS observed in the Saguenay Fjord are generally at the higher end of the range of values seen elsewhere. Other studies have found $DMSP_d$ to DMS ratios of 4 (North Sea, Turner et al. 1988), 0.08–6 (east coast of the United States, Iverson et al. 1989), 3–39 (Antarctic Peninsula region, Turner et al. 1995), and 1–42 (western Mediterranean Sea, Simó et al. 1997). Given that up to 70% of the $DMSP_d$ removal can occur via pathways that do not lead to the formation of DMS (Malin and Kirst 1997), the high ratios of $DMSP_d$ to DMS, together with very low DMS concentrations, would suggest that bacterial consumption of these compounds may be higher in this fjord than in other marine environments. As such, this may have contributed to the low levels of dissolved dimethylated sulfur observed.

$DMSO_d$ was not detected at any of the stations occupied in the Saguenay Fjord. The absence of DMSO in the water column of the fjord is atypical for aquatic environments in general. Normally, DMSO is the most important form of dimethylated sulfur in the marine environment (de Mora et al. 1996; Hatton et al. 1996; Lee and de Mora 1996). Initially, it was thought that the relatively high detection limit for DMSO using the direct technique might have contributed to DMSO not being detected. With the detection limit of the system used for this study, $DMSO_d$ concentrations could have been an order of magnitude greater than DMS concentrations, yet remained undetected. Thus, a cross section of samples, representing 42% of the total number of samples taken, was reanalysed using a modified version of the sodium borohydride reduction technique (Simó et al. 1997). The detection limit for samples measured with this method was 16 pmol·L^{-1} rather than 2500 pmol·L^{-1} with the direct method. This is comparable with the detection limit of the system outlined earlier in the Materials and methods section that was used to measure DMS and DMSP concentrations. Despite the increased sensitivity of the reduction method, $DMSO_d$ was still not observed in any of the samples analysed.

This study represents one of the few occasions where $DMSO_d$ has not been found to be one of the most important dissolved forms of dimethylated sulfur in the marine environment. The most likely explanation for this observation was the very low DMS concentrations observed coupled with the very shallow photic zone. In the Saguenay Fjord, the depth of the photic zone rarely exceeds 7 m (Table 1) due to the presence of high quantities of suspended particulate material and dissolved organic material. While dissolved organic material is an excellent source of radical species that can initiate the oxidation of DMS to DMSO, in this environment, the high concentrations of dissolved organic material severely limit the penetration of light. Photochemical oxidation of DMS via hydroxyl and dibromine radicals is a known pathway for the formation of DMSO (Schöneich et al. 1993). Thus, in the case of the Saguenay Fjord, the photochemical formation of DMSO is effectively limited to a near-surface zone where the low salinity would limit dibromine radical production and the atmospheric evasion of DMS is the most important process controlling the removal of DMS from the water column (Kieber et al. 1996). Such a scenario would result in low concentrations of dissolved DMSO.

Although $DMSO_d$ was not observed, $DMSO_p$ was measured in filtered phytoplankton samples. While the measurement of DMSO in marine phytoplankton is new, DMSO concentrations have been previously determined in vegetables and fruits (Pearson et al. 1981) and in human blood (Martin 1987). These results imply that DMSO may be produced biosynthetically. Andreae (1980) theorized that DMSO may occur as a byproduct of algal activity and is not necessarily related to, or uniquely a result of, the decay of biomass. More recently, some speculation has been made as to the presence of biosynthetically produced DMSO in the marine environment (de Mora et al. 1996; Lee and de Mora 1996), and preliminary results have been reported that support this speculation (Simó et al. 1998).

The reasons as to why DMSO would be present intracellularly are not well defined, and unfortunately, this data set is too small to provide concrete evidence as to the role of DMSO in marine organisms. Some potential roles that intracellular DMSO could fulfil include cryoprotectant, cryoosmoregulator, and free-radical scavenger. Phytoplankton could also produce DMSO to modify intracellular electrolyte fluids to allow more favourable reaction conditions for met-

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abolic processes. A more extensive discourse on the potential biological functions of DMSO and possible metabolic pathways for the formation of intracellular DMSO can be found elsewhere (Lee and de Mora 1999). The presence of DMSO within cells has several implications for the marine sulfur cycle. Firstly, it represents a formation pathway for dissolved DMSO that does not involve water column DMS. Secondly, factors such as zooplankton grazing and cell senescence, which influence the $DMSP_d$ pool through the release of intracellular DMSP, could similarly affect DMSO. Finally, DMSO can rapidly permeate through biological membranes. Phytoplankton, which are in continuous contact with seawater, may be unable to prevent any permeative loss, or gain, of DMSO due to concentration gradients set up between intracellular fluids and the extracellular seawater.

$DMSO_p$ concentrations ranged from below the limit of detection to 110 nmol·L^{-1} and on occasions, concentrations were one to two orders of magnitude greater than $DMSP_p$ concentrations. In comparison, Simó et al. (1998) found that $DMSO_p$ levels (2.7–16 nmol·L^{-1}) were typically an order of magnitude lower that $DMSP_p$ levels (7.1–340 nmol·L^{-1}) at a site in the North Sea. While some correlation appears to exist between $DMSO_p$ and $DMSP_p$ (Fig. 2) in the Saguenay Fjord, the ratio of these two compounds is not consistent. For example, the average $DMSO_p$ to $DMSP_p$ ratio at station MOF (26:1) is less than that at station BHH (112:1). The same trend can be observed for both vertical profiles and temporal series at a depth of 2 m. This is primarily due to increased levels of $DMSP_p$ at station MOF, with average values for $DMSO_p$ remaining relatively constant throughout the fjord (Table 2). This would suggest that the ability to biosynthesize DMSO might be present in more planktonic organisms than the ability to synthesize DMSP. Based on results from culture experiments involving *Amphidinium carterae*, *Emiliania huxleyi*, and *Pleurochrysis carterae*, a similar finding has been reported by Simó et al. (1998).

In contrast with the positive correlation usually observed for $DMSP_p$ and Chl *a*, a significant negative correlation was observed between $DMSO_p$ and total Chl *a* ($r = -0.473$, $p \leq 0.05$) during studies of temporal variability at station MOF. A weakly significant correlation was also observed between $DMSO_p$ and Chl *a* for the <5-μm fraction ($r = -0.429$, $p = 0.067$). An explanation for this observation may be that as Chl *a* levels and photosynthetic activity increase, more free radicals are generated by photosynthetic processes. If DMSO is produced to act as an intracellular free-radical scavenger (vide supra), then loss of this compound through scavenging may be a response to the presence of increased levels of free radicals associated with increased photosynthetic activity. Thus, a negative correlation would be observed. In comparison with other studies indicating that intracellular DMSP concentrations are greater than those of DMSO (Simó et al. 1998; R. Haase et al., unpublished data), the results obtained here demonstrate that concentrations of DMSO present in the particulate phase may be comparable with those of intracellular DMSP. These results confirm recent speculations and observations that DMSO is present in particulate material. The biosynthesis of DMSO by phytoplankton represents a pathway for the formation of dissolved DMSO that, until very recently, has gone unrecognized in aquatic ecosystems.

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References

Andreae, M.O. 1980. Dimethylsulfoxide in marine and freshwaters. Limnol. Oceanogr. **25**: 1054–1063.

Bratbak, G., Levasseur, M., Michaud, S., Cantin, G., Fernandez, E., Heimdal, B.R., and Heldal, M. 1995. Viral activity in relation to *Emiliania huxleyi* blooms: a mechanism of DMSP release? Mar. Ecol. Prog. Ser. **128**: 133–142.

Cantin, G., Levasseur, M., Gosselin, M., and Michaud, S. 1996. Role of zooplankton in the mesoscale distribution of surface dimethylsulfide concentrations in the Gulf of St. Lawrence, Canada. Mar. Ecol. Prog. Ser. **141**: 103–117.

Chanut, J.-P., and Poulet, S.A. 1979. Distribution des spectres de tailles des particules en suspension dans le fjord du Saguenay. Can. J. Earth Sci. **16**: 240–249.

De Ladurantaye, R., Therriault, J.-C., Lacroix, G., and Côté, R. 1984. Processus advectifs et répartition du zooplancton dans un fjord. Mar. Biol. **82**: 21–29.

de Mora, S.J., Grout, A., and Shooter, D. 1990. The analysis of reduced sulfur gases in air and melt waters by gas chromatography at Bratina Island, 78°S. N.Z. Antarct. Rec. **10**: 12–21.

de Mora, S.J., Lee, P., Shooter, D., and Eschenbruch, R. 1993. The analysis and importance of dimethylsulfoxide in wine. Am. J. Enol. Vitic. **44**: 327–332.

de Mora, S.J., Lee, P.A., Grout, A., Schall, C., and Heumann, K.G. 1996. Aspects of the biogeochemistry of sulfur in glacial melt water ponds on the McMurdo Ice Shelf, Antarctica. Antarct. Sci. **8**: 15–22.

Drainville, G. 1968. Le fjord du Saguenay: 1. Contribution à l'océanographie. Nat. Can. **95**: 809–855.

Dyer, K.R. 1973. Estuaries: a physical introduction. John Wiley & Sons, London, U.K.

Gratton, Y., Couture, M., and Lefaivre, D. 1993. Océanographie physique du fjord du Saguenay. *In* Le fjord du Saguenay: un milieu exceptionnel de recherche. *Edited by* J.-M. Sévigny and C.M. Couillard. Rapp. manusrit can. sci. halieut. aquat. n° 2270.

Hatton, A.D., Malin, G., Turner, S.M., and Liss, P.S. 1996. DMSO. A significant compound in the biogeochemical cycle of DMS. *In* Biological and environmental chemistry of DMSP and related sulfonium compounds. *Edited by* R.P. Kiene, P.T. Visscher, M.D. Keller, and G.O. Kirst. Plenum Press, New York. pp. 405–412.

Iverson, R.L., Nearhoof, F.L., and Andreae, M.O. 1989. Production of dimethylsulfonium propionate and dimethylsulfide by phytoplankton in estuarine and coastal waters. Limnol. Oceanogr. **34**: 53–67.

© 1999 NRC Canada

Kieber, D.J., Jiao, J., Kiene, R.P., and Bates, T.S. 1996. Impact of dimethylsulfide photochemistry on methyl sulfur cycling in the equatorial Pacific Ocean. J. Geophys. Res. **101**: 3715–3722.

Lee, P.A., and de Mora, S.J. 1996. DMSP, DMS and DMSO concentrations and temporal trends in marine surface waters at Leigh, New Zealand. *In* Biological and environmental chemistry of DMSP and related sulfonium compounds. *Edited by* R.P. Kiene, P.T. Visscher, M.D. Keller, and G.O. Kirst. Plenum Press, New York. pp. 391–404.

Lee, P.A., and de Mora, S.J. 1999. Intracellular DMSO in unicellular marine algae: speculations on its origin and possible biological role. J. Phycol. **35**: 8–18.

Levasseur, M., Keller, M., Bonneau, E., D'Amours, D., and Bellows, W.K. 1994. Oceanographic basis of a DMS-related Atlantic cod (*Gadus morhua*) fishery problem: blackberry feed. Can. J. Fish. Aquat. Sci. **51**: 881–889.

Levasseur, M., Sharma, S., Cantin, G., Michaud, S., Gosselin, M., and Barrie, L. 1997. Biogenic sulfur emissions from the Gulf of Saint Lawrence and assessment of its impact on the Canadian east coast. J. Geophys. Res. **102**: 28 025 – 28 039.

Liss, P.S., Hatton, A.D., Malin, G., Nightingale, P.D., and Turner, S.M. 1997. Marine sulphur emissions. Philos. Trans. R. Soc. Lond. B, Biol. Sci. **352**: 159–169.

Lovejoy, C., Vincent, W.F., Frenette, J.-J., and Dodson, J.J. 1993. Microbial gradients in a turbid estuary: application of a new method for protozoan community analysis. Limnol. Oceanogr. **38**: 1295–1303.

Malin, G., and Kirst, G.O. 1997. Algal production of dimethyl sulfide and its atmospheric role. J. Phycol. **33**: 889–896.

Malin, G., Turner, S.M., and Liss, P.S. 1992. Sulfur: the plankton/climate connection. J. Phycol. **28**: 590–597.

Martin, W. 1987. Natürliches vorkommen von dimethylsulfoxid (DMSO) und dimethylsulfon ($DMSO_2$) im menschlichen organismus. *In* DMSO, Internationaler DMSO Workshop. *Edited by* S.W. Jacob and J.E. Kappel. W. Zuckschwerdt Zerlag, München. pp. 71–77.

Parsons, T.R., Maita, Y., and Lalli, C.M. 1984. A manual of chemical and biological methods for seawater analysis. Pergammon Press, Toronto, Ont.

Pearson, T.W., Dawson, H.J., and Lackey, H.B. 1981. Natural occurring levels of dimethyl sulphoxide in selected fruits, vegetables, grains, and beverages. J. Agric. Food Chem. **29**: 1089–1091.

Schafer, C.T., Smith, J.N., and Côté, R. 1990. The Saguenay Fjord: a major tributary to the St. Lawrence Estuary. *In* Oceanography of a large scale estuarine system: the St. Lawrence. *Edited by* M.I. El-Sabh and N. Silverberg. Springer-Verlag, New York. pp. 378–420.

Schöneich, C., Aced, A., and Asmus, K.-D. 1993. Mechanism of oxidation of aliphatic thioethers to sulfoxides by hydroxyl radicals. The importance of molecular oxygen. J. Am. Chem. Soc. **115**: 11 376 – 11 383.

Shooter, D., de Mora, S.J., Grout, A., Wylie, D.J., and Zhi-Yun, H. 1992. The chromatographic analysis of reduced sulfur gases in Antarctic waters following the pre-concentration onto Tenax. Int. J. Environ. Anal. Chem. **47**: 239–249.

Simó, R., Grimault, J.O., and Albaigés, J. 1997. Dissolved dimethylsulphide, dimethylsulphoniopropionate and dimethylsulphoxide in western Mediterranean waters. Deep-Sea Res. **44**: 929–950.

Simó, R., Hatton, A.D., Malin, G., and Liss, P.S. 1998. Particulate dimethyl sulphoxide in seawater: production by microplankton. Mar. Ecol. Prog. Ser. **167**: 291–296.

Therriault, J.-C., de Ladurantaye, R., and Ingram, R.G. 1984. Particulate matter exchange across a fjord sill. Estuarine Coastal Shelf Sci. **18**: 51–64.

Throndsen, J. 1978. Preservation and storage. *In* Phytoplankton manual. *Edited by* A. Sournia. UNESCO, Paris. pp. 69–74.

Tsuji, T., and Yanagita, T. 1981. Improved fluorescent microscopy for measuring the standing stock of phytoplankton including fragile components. Mar. Biol. **64**: 307–211.

Turner, S.M., Malin, G., Liss, P.S., Harbour, D.S., and Holligan, P.M. 1988. The seasonal variation of dimethyl sulfide and dimethylsulfoniopropionate concentrations in nearshore waters. Limnol. Oceanogr. **33**: 364–375.

Turner, S.M., Nightingale, P.D., Broadgate, W., and Liss, P.S. 1995. The distribution of dimethyl sulphide and dimethylsulphoniopropionate in Antarctic waters and sea ice. Deep-Sea Res. **42**: 1059–1080.

Utermöhl, H. 1958. Zur Vervolkommnung der quantitativen Phytoplankton-Methodik. Mitt. Int. Ver. Theor. Angew. Limnol. **9**.

Wilkinson, L., Hill, M., Welna, J.P., and Birkenbeud, G.K. 1992. SYSTAT for Windows: statistics, version 5 edition. SYSTAT Inc., Evanston, Ill.

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Role of copepod-dominated meiofauna in the nitrification process of a cold marine mesocosm

Serge Parent and Antoine Morin

Abstract: Large populations of copepod-dominated meiofauna are found in the sand filters of the St. Lawrence marine mesocosm at the Montreal Biodome. Experiments were conducted in heterotrophic microcosms to quantify how populations of micro- and meiofaunal organisms affect ammonia oxidation (nitrition) and nitrite oxidation (nitration) using apparent nitrition rate (ANiR) and apparent nitration rate (ANaR) as proxies. ANiR and ANaR were not related to ciliate density. Meiofauna had no effect on ANiR, but a significant relationship between ANaR and meiofaunal biomass was observed, which varied with the particulate organic nitrogen (PON) content of the sediment. The relationship was negative at low PON and positive at high PON. These results suggest a direct negative action by predation on nitrifying bacteria and an indirect positive action by reducing competitors through grazing of heterotroph bacteria. The negative effect of 1 g meiofauna·m^{-2} at low PON (–20% of $ANaR_0$, i.e., ANaR without meiofauna) is much smaller than its positive effect at high PON (+172 to +571% of $ANaR_0$). Copepod-dominated meiofaunal biomasses less than 0.16 g·m^{-2} increase two to five times the nitrification rate in heterotrophic habitats rich in PON.

Résumé : Les filtres à sable du mésocosme du Saint-Laurent marin du Biodôme de Montréal renferment d'importantes populations de méiofaune dominées par les copépodes. Des expériences ont été menées dans des microcosmes hétérotrophiques pour mesurer l'effet de cette méiofaune et de la microfaune sur l'oxydation de l'ammoniac (nitrition) et des nitrites (nitration) via le taux apparent de nitrition (TANi) et le taux apparent de nitration (TANa). Le TANi et le TANa n'ont pas été corrélés à l'abondance des ciliés. La méiofaune n'a pas eu d'effet sur le TANi mais il existe une relation entre la méiofaune et le TANa. Cette relation a varié selon la teneur en azote organique particulaire (APO) des sédiments : elle a été négative pour un APO faible et positive pour un APO élevé. Les résultats suggèrent une action directe négative de prédation des bactéries autotrophes conjugée à une action indirecte positive de réduction des compétiteurs par le broutage des bactéries hétérotrophes. L'effet négatif de 1 g méiofaune·m^{-2} à des AOP faibles (–20% de $TANa_0$, c.-à-d., le TANa en l'absence de méiofaune) est plus faible que son effet positif à des AOP élevés (+172 à +571% de $TANa_0$). A moins de 0,16 g·m^{-2}, une méiofaune dominée par les copépodes accroît de deux à cinq fois le taux de nitrification dans les habitats hétérotrophiques riches en AOP.

Introduction

Nitrification, the process by which ammonia is converted into nitrate, is of utmost concern in closed systems, such as public aquariums and mesocosms (Stoskopf 1993). In these managed environments, impairment of nitrification usually results in a buildup of ammonia that threatens the health of the fish and invertebrates on display. Ammonia is produced by the digestion of organic matter by heterotrophic bacteria. It is further oxidized into nitrite, and into much less toxic nitrate, by chemoautotrophic bacteria (Spotte 1991). Biological filtration systems for large marine aquaria, such as the St. Lawrence mesocosm (SLM) of the Montreal Biodome, depend on this bacterial activity to maintain life support.

The oxidation of ammonia into nitrite (nitrition) is mainly performed by *Nitrosomonas* whereas *Nitrobacter*, *Nitrococcus*, and *Nitrospina* (Watson et al. 1989; Spotte 1991) are largely responsible for the oxidation of nitrite into nitrate (nitration). Nitrifying bacteria are not attached to the substrate as individuals cells but exist as colonies within self-secreted layers of biological films that coat sand and gravel (Spotte 1991). The nitrification capacity of an aerobic biofilm is determined by the number and activity of nitrifying bacteria in it (Lee and Welander 1994).

Bacteria are grazed by a large variety of organisms such as flagellates, ciliates, meiofauna, (benthos smaller than 1 mm such as rotifers and nematodes), and macrofauna (e.g., oligochetes and snails). While many studies have shown that the mineralization rate of organic matter is affected by protozoans (Johannes 1965; Pomeroy 1970; Barsdate et al. 1974) and by meiofauna (Tenore et al. 1977; Findlay and Tenore 1982; Alkemade et al. 1992), the effects of protozoans and meiofauna on nitrification have received comparatively little attention (Lee and Welander 1994). Verhagen and Laanbroek (1992) found that flagellates preyed only on free nitrifying cells and that, in the presence of flagellates,

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Table 1. Summary of the nitrification experiments and organic matter content of the mesocosm sand filters.

	Nitrition experiment			
	NH_3Try	NH_3Det	Sand filter	
Number of microcosms	8	8		
Organic substrate	Tryptone	Detritus		
Stimulation of nitrition	Yes	Yes		
Impoverishment in meiofauna	No	Yes		
Organic content ($g \cdot m^{-2}$)	112–120	281	231–332	
C:N ratio	4.4–12	7.9	6.3	
%N	0.38–0.62	2.15	2.00	
PON content ($g \cdot m^{-2}$)	0.46–0.78	6.0	4.6–6.6	
Nitrate (mg NO_3-$N \cdot L^{-1}$)	26–40	20–40	>100	
	Nitration experiment			
	NO_2Try1	NO_2Fis2	NO_2Fis3	NO_2Fis4
Number of microcosms	8	7	10	16
Organic substrate	Tryptone	Fishmeal	Fishmeal	Fishmeal
Stimulation of nitrification	Yes	No	Yes	Yes
Meiofauna enrichment	No	Yes	Yes	Yes
Organic content ($g \cdot m^{-2}$)	112–120	265	265	204
C:N ratio	4.4–12	8.2	8.2	7.6
%N	0.38–0.62	2.40	2.40	1.31
PON content ($g \cdot m^{-2}$)	0.46–0.78	5.2–7.8	5.2–7.8	1.8–4.6
Nitrate (mg NO_3-$N \cdot L^{-1}$)	26–40	9–22	9–22	5–19

the number of nitrifying bacteria declined but not the rate of nitrate production. However, the effect of grazing by protozoans on biofilms containing attached nitrifying bacteria is still unclear. Many phagotrophs graze on only a few bacterial species (Curds 1975; Sudo and Aiba 1984). Griffiths (1989) did not find any protozoan that would graze specifically on nitrifiers and attributed this to the large agglomerative and strongly adhesive growth form of nitrifiers that makes them more difficult to ingest than single cells.

Recent studies on freshwater trickling filters have shown that meiofauna reduces nitrification (Parker et al. 1989; Andersson et al. 1994; Lee and Welander 1994). Predators, such as rotifers, may have a strong negative impact on nitrification in aerobic biofilm processes, at least in systems not limited by oxygen or ammonium transfer. This is probably because grazing by microfauna has a greater negative effect on the slow-growing chemoautotrophs than on the fast-growing heterotrophs (Lee and Welander 1994).

The present study was carried out to test whether the meiofauna living in the sand filters of the SLM negatively affect nitrification. The SLM is a closed aquatic system that holds 3×10^6 L of cold (10°C) artificial seawater (28–30‰). It exhibits fish, invertebrates, and seabirds from the Gulf of St. Lawrence. The water treatment system consists of six sand filters 3.6 m in diameter, a 200-m^3 trickling filter, and an ozonation system. All detritus ends up in the sand filters where most of the meiofauna is located (S. Parent, unpublished data). Experiments were conducted in laboratory model systems (heterotrophic microcosms) containing synthetic seawater and fixed quantities of organic substrates. Protozoan abundances were controlled and meiofaunal abundances manipulated prior to addition of ammonia or nitrite. Known quantities of ammonia and nitrite were added and residual concentrations were measured at regular time intervals. Rates of nitrition and nitration were then estimated and correlated with protozoan numbers and meiofaunal biomasses.

Materials and methods

Experimental setup

The experiments were performed in heterotrophic microcosms. These were 20-L all-glass aquariums (778 cm^2) containing 13.5 L of water. Each microcosm was equipped with an undergravel filter and two airlifts (filtration rate 41–82 $L \cdot m^{-2} \cdot min^{-1}$, turnover rate about 2–4 min). The bottom of each microcosm was covered with 4 cm of filter sand. A shallow sandbed and a uniform yet rapid flow of oxygen-saturated water from the top to the bottom of the sandbed insured constant oxygen saturation in the porewater of the sediment. Seawater 28–30‰ was prepared using Instant Ocean® sea salts. The microcosms were maintained in a cold room at 10°C and in the dark to prevent algal growth.

Nitrition experiments

Two nitrition experiments were carried out by adding 1 mg NH_3-$N \cdot L^{-1}$ to selected microcosms (Table 1). The source of organic matter for heterotrophic bacteria, ciliates, and meiofauna was tryptone or detritus. Tryptone and detritus, differing in particle size and nitrogen content, were used to generate different nitrifying activities and to observe the effect of meiofauna on different nitrition rates. Ammonia and nitrite levels were zero at the start of each experiment, and pH was similar (8.07–8.45).

Eight microcosms (denoted NH_3Try) were given tryptone for 4 months before the experiment. The tryptone used was trypticase soy broth (soybean–casein digest broth, Difco 1984). When put in seawater, tryptone quickly dissolves and becomes dissolved organic matter. It contains 8.7% nitrogen and 33.2% carbon, for a C:N ratio of 4.4 (Table 1). Nitrition was stimulated with two dosages of 1 ppm NH_4Cl-N given 32 and 23 days prior to the experiment.

A second set of eight microcosms (denoted NH_3Det) were set up 73 days before the start of the experiment. One sand filter clogged with detritus (uneaten food, feces, periphyton, dead invertebrates,

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etc.) from the SLM was opened, and for each microcosm, 5.7 kg of the top 2.5 cm of wet sand was collected using a wide aluminium pan with one end sectioned. The top 2.5 cm of sand contained 281 g dry mass detritus·m^{-2} (Table 1). The detritus had a mean particle size of 400 µm and contained 2.2% nitrogen and 14.6% carbon, for a C:N ratio of 7.9 (Table 1). The weight of sand corresponded to 4 cm of sand in the experimental microcosms. From day –68 to day –54, meiofauna was depressed in four microcosms using three doses of 10 ppm nystatin and 10 ppm cycloheximide. These two substances are inhibitory to eucaryotic organisms (Lee and Welander 1994). Nitrition was stimulated with one dose of 1 mg NH_3-N·L^{-1} given 6 days prior to the experiment.

In all experiments, 27 mL of 0.0355 M NH_4Cl was added to each microcosm at $t = 0$, and residual ammonia concentrations were measured at regular time intervals.

Nitration experiments

Four nitration experiments were carried out by adding 1 mg NO_2-N·L^{-1} in selected microcosms. The source of organic matter for heterotrophic bacteria, ciliates, and meiofauna was tryptone in the first experiment (NO_2Try) and fishmeal in the other three (NO_2Fis2–4). The source of organic matter and stimulation of nitrification were varied among experiments to generate different nitrifying activities and to observe the effect of meiofauna on different nitration rates. Ciliates were controlled with doses of 10 ppm nystatin and 10 ppm cycloheximide given about 1 month before each experiment. Ammonia and nitrite levels were below detection levels at the start of each experiment, and pH was similar (8.13–8.35).

NO_2Try was done using eight microcosms, and nitrification was stimulated with two doses of 1 mg NH_3-N·L^{-1} given 12 and 3 days before the experiment (Table 1). NO_2Fis2–4 were conducted using seven, 10, and 16 microcosms, respectively. All microcosms were given tryptone or fishmeal for at least 4 months. The fishmeal used was redfish (*Sebastes marinus*), produced locally, with a particle size of 100–150 µm. It contained 11.5% nitrogen and 45.7% carbon (Table 1). Nitrification was not stimulated in NO_2Fis2, but it was in NO_2Fis3 and NO_2Fis4 with weekly doses of 0.5 or 1.0 mg NO_2-N·L^{-1}.

Meiofauna was not controlled in the tryptone experiment whereas it was enriched in one or more of the microcosms 2 weeks before each fishmeal experiment (Table 1). Meiofaunal abundances were manipulated through additions of meiofauna from the sand filters. Meiofauna from the mesocosm sand filters was collected at the time of backwash using a Wisconsin-style plankton net with 80-µm mesh. Small floating 300-µm sieves covered with ice were then used to extract meiofauna from the detritus.

In all experiments, 10 mL of 0.1 M $NaNO_2$ was added to each microcosm at $t = 0$, and residual nitrite concentrations were measured at regular time intervals.

Biological determinations

Organisms could not be sampled during the experiments without disturbing the microcosms, so microfauna and meiofauna were measured no more than 10 days prior to or after each experiment.

Because meiofauna is patchy in its distribution (Findlay 1981), the sand was raked before each sampling so as to obtain a better estimate of the meiofaunal abundance with the least effort. Usually, five samples, but sometimes up to eight, of the 0- to 2.5-cm layer were randomly taken using a 10-mL syringe (1.63 cm^2, 4 cm^3 of sand). Meiofauna and microfauna were separated in a 50-mL cylinder using the decantation method described by Pfannkuche and Thiel (1988) and then collected on a 20-µm sieve. By closely examining 1 cm^3 of residue of randomly chosen samples, decantation was estimated to be at least 95% effective in extracting meiofauna. All animals were counted alive. A different set of samples was taken to assess the standard weight of each taxon: length and width of specimens were taken and then converted into weights using conversion factors provided by Feller and Warwick (1988). These standard weights were then combined with the abundance results to establish the meiofaunal biomass in each sample. The sand of all samples, but not the meiofauna, was put back into the microcosms. Less than 1% of the meiofauna was so removed before or after each experiment.

Three groups of protozoans were observed: ciliates, foraminiferans, and heliozoans. Only ciliates were quantified. Ciliate populations were found by Fenchel (1967, 1978) to outnumber all other fauna in well-mixed medium (100–300 µm) sands with little interstitial silt and clay, as is the case here. They were therefore used as an index of protozoan abundance.

Chemical analysis

Total ammoniacal nitrogen (TAN) was measured using the ammonia-selective electrode method, and nitrite was measured using the colorimetric method (APHA et al. 1992). Detection levels of these methods are 0.03 mg NH_3-N·L^{-1} and 0.02 mg NO_2-N·L^{-1}. The levels of TAN or nitrite of each microcosm were then plotted against time. Levels of TAN higher than 0.2 mg NH_3-N·L^{-1} and levels of nitrite higher than 0.1 mg NO_2-N·L^{-1} were found to decrease linearly with time. Nitrition and nitration rates were estimated by regressing those levels against time. Total carbon and nitrogen content of organic matter was analyzed on a Carlo-Erba CHN analyzer. Organic matter content was determined by loss on ignition at 400–450°C.

Experiments in which measurements of residual concentrations in the water are used to estimate process rates generate "apparent" reaction rates because the rates so obtained are a composite of all reactions rates occurring within the microcosm (Parker et al. 1989), including nitrification by chemoautotrophic bacteria, assimilation by heterotrophic bacteria, and mineralization of organic matter (Verhagen and Laanbroek 1992). We therefore used the expressions apparent nitrition rate (ANiR) and apparent nitration rate (ANaR) (see Parker et al. 1989, Andersson et al. 1994).

Statistical analysis

The relationships between ANiR or ANaR and microfaunal abundance (number per 10 cm^2) or meiofaunal biomass (grams per square metre) were quantified by linear regression analysis. For each microcosm, the biomass was set as the arithmetic mean of the readings (generally five readings each time, but sometimes up to eight). Regression analyses between apparent nitrification rate (ANR) and its standard error showed ANiR and ANaR to be homoscedastic.

Results

Meiofaunal composition and abundance

Microcosms

The meiofauna was composed of seven taxa: harpacticoid copepods, nematodes, rotifers, polychaetes, turbellarians, mites (Acari), and gastrotrichs. The harpacticoid copepods dominated both in number (87%) and in biomass (98%) with three species present: *Pseudonychocamptus proximus*, *Tisbe* sp. (*furcata*?), and *Paramphiascella* sp. (*fulvofasciata*?). *Pseudonychocamptus* largely dominated the group with over 90% of the specimens. Nematodes and rotifers accounted for only 4.6 and 8%, respectively, of the specimens and 0.8 and 0.5%, respectively, of the biomass, while other taxa accounted for less than 1% of the specimens and the biomass. Meiofaunal biomass varied from 0 to 1.84 g·m^{-2} in the

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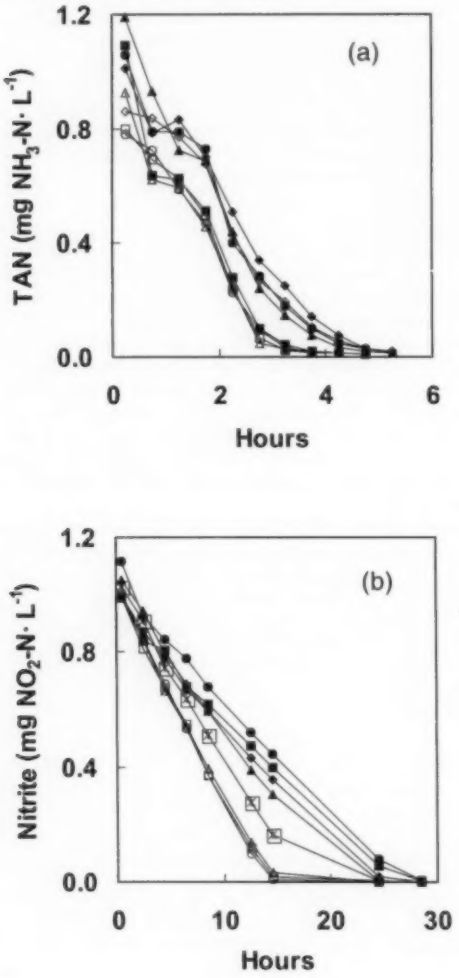
Fig. 1. Reduction of (*a*) TAN during the NH_3Det experiment and (*b*) nitrite during the NO_2Try experiment. The linear regression adjusted well the data between 0.2 and 1.0 mg NH_3-N·L^{-1} and between 0.1 and 1.0 mg NO_2-N·L^{-1}. Different symbols represent the separate microcosms for each experiment.



nitrition experiments and from 0 to 2.31 g·m^{-2} in the nitration experiments.

Sand filters

The composition of the sand filter meiofauna is the same as in the microcosms, except for a few hydrozoan polyps that were absent in the microcosms. Meiofaunal biomass in the 0- to 2.5-cm layer of the sand filters varied from 20 to 40 g·m^{-2} during the experiments. Recent sampling (July 1997) has revealed that meiofauna is quite abundant throughout the sand column, with values close to 1 g·m^{-2} in core increments of 2.5 cm (or grams per 25 dm^3) at least down to 25 cm (S. Parent, unpublished data). Meiofaunal biomasses in the microcosms were therefore much less than what was found in the sand filters.

Amount and C:N ratio of organic matter in the substrate

For NH_3Try and NO_2Try, no sampling was done at the time of the experiment for the organic matter content of the substrate and its C:N ratio. Organic matter content was measured 3 months later. Based on the additions of tryptone 35 days before and the mineralization rates of tryptone (Parent and Morin 1999), the organic content of the substrate was estimated to be between 112 and 120 g·m^{-2} and the C:N ratio to be between 4.4 and 12 during the experiment (Table 1).

For the NH_3Det experiment, organic matter content was measured 3 days before the experiment and the C:N ratio 1 week after. The organic matter content was 281 g·m^{-2} (SE = 18.2, n = 8) and the C:N ratio was 7.9 (SE = 0.25, n = 8) (Table 1). For the three fishmeal nitration experiments, both parameters were measured before NO_2Fis2, NO_2Fis3, and NO_2Fis4. The organic matter content was 265 g·m^{-2} (SE = 19.9, n = 8) for NO_2Fis2 and NO_2Fis3 and 204 g·m^{-2} (SE = 34.5, n = 16) for NO_2Fis4. The C:N ratio was 8.2 (SE = 0.40, n = 8) for NO_2Fis2 and NO_2Fis3 and 7.6 (SE = 0.52, n = 16) for NO_2Fis4 (Table 1).

ANiR

Ammonia concentrations declined linearly between 1.0 and 0.20 ppm in the NH_3Det microsoms (Fig. 1*a*) and in the NH_3Try microcosms. Only those results were used to calculate the ANiR of each microcosm through linear regression. Standard error of regression slopes was 4–18% of the slope. It took 8–21 h to reduce the ammonia level from 1.0 to 0.2 ppm in the tryptone experiment, and ANiR varied from 7 to 22 mg·m^{-2}·h^{-1} (Fig. 2). In the detritus experiment, it took 3–5 h to reduce the ammonia level from 1 to 0.2 ppm (Fig. 1*a*), and ANiR varied from 58 to 90 mg·m^{-2}·h^{-1} (Fig. 2).

ANaR

Nitrite concentrations declined linearly between 1.0 and 0.1 ppm in the NO_2Try microcosms (Fig. 1*b*) and in the microcosms of the three fishmeal experiments. Only those results were used to calculate the ANaR of each microcosm through linear regression. Standard error of regression slopes was 0.5–6% of the slope. It took 12–24 h to reduce nitrite from 1.0 to 0.1 ppm in the tryptone experiment, and ANaR varied from 7.0 to 13.2 mg·m^{-2}·h^{-1} (Fig. 3). In the three fishmeal experiments, nitrite was reduced from 1.0 to 0.1 ppm over a period of 4–13 h, and ANaR varied from 10.1 to 33.2 mg·m^{-2}·h^{-1} (Fig. 3).

Effect of ciliate density and meiofaunal biomass on ANR

ANiR was not correlated with ciliate density or with meiofaunal biomass (Table 2). In all four nitration experiments, ANaR was not correlated with ciliate density. No significant relationship was found between ANaR and meiofaunal biomass in NO_2Fis4, but a significant relationship was found in NO_2Try and in NO_2Fis2 and NO_2Fis3 (Table 2). Correlation tests were also done for subsets of NO_2Fis3 and NO_2Fis4 (these subsets are described below): no relationship was found between ciliate density and ANaR (Table 2).

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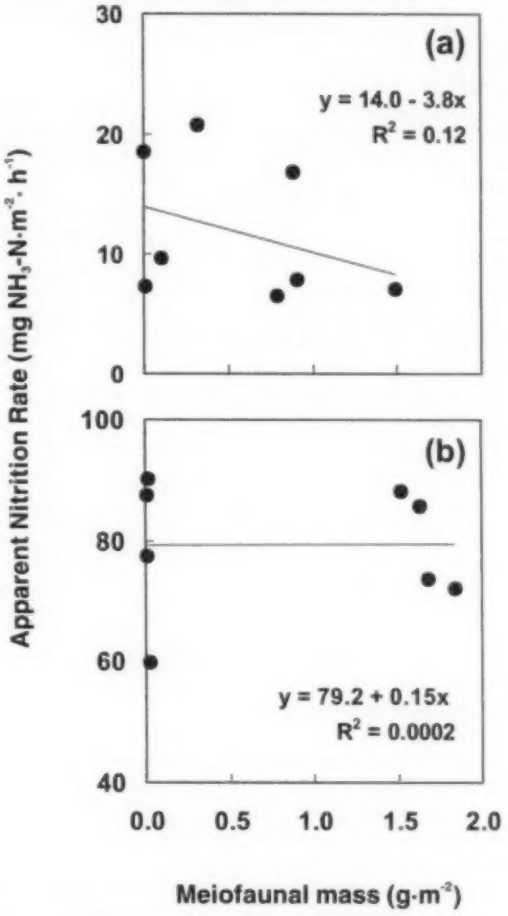

Fig. 2. Plot of ANiR versus meiofaunal biomass in the (*a*) tryptone experiment and (*b*) detritus experiment. There were no correlations between ANiR and meiofaunal biomass.

Linear regression models

No correlation was found between ANiR and meiofaunal biomass, but the relationship between ANaR and meiofaunal biomass varied: it was significantly negative in the NO_2Try experiment and significantly positive in NO_2Fis2 and NO_2Fis3 (Table 3). Meiofaunal biomass explained between 41 and 85% of the variation in ANaR in these nitration experiments (Fig. 3). The models show that 1 g·m^{-2} of meiofauna reduced ANaR by 20% when there is no meiofauna in the tryptone experiment but increased it in NO_2Fis2 by 456% and in NO_2Fis3 by 64%. The apparent effect was nil in NO_2Fis4.

The effect of biomass was seven times stronger in NO_2Fis2 than in NO_2Fis3 under similar conditions (Table 1) but over a different range of meiofaunal biomasses (Fig. 3). Data from NO_2Fis3 were reanalyzed separately for biomasses similar to those of NO_2Fis2 (≤0.16 g·m^{-2}, n = 4) and larger than 0.16 g·m^{-2} (n = 6). The regression for the subset of biomasses similar to those of NO_2Fis2 was significant and showed an increase in $ANaR_0$ (571%) similar to the one observed in NO_2Fis2 (456%) (Table 3). Similarly, NO_2Fis4 data were also subdivided into two subsets but with different biomass ranges to account for a poorer organic substrate (Tables 1 and 3). The subset for biomasses less than 0.12 g·m^{-2} was significant and also showed a strong increase in $ANaR_0$ (970%).

To better understand what controls the variation in ANaR, all results from the four nitration experiments (n = 41) were pooled together and ANaR was regressed against meiofaunal mass, ciliates, organic matter content of the sediment (ORG), nitrate, percentage of nitrogen (%N), and the C:N ratio of organic matter. ORG and %N were chosen because heterotrophic bacterial activity is related to the amount of organic matter in the sediment and to its nitrogen content (DeAngelis 1992). The square root of ANaR and of meiofaunal biomass was used for the regressions so as to linearize the relationship, to stabilize the variance of the residuals, and to reduce the influence of the few most extreme observations (Table 4). ANaR was not correlated with ciliates (R^2 = 0.07) or with C:N ratio (R^2 = 0.07), but it was correlated with nitrate (R^2 = 0.22), ORG (R^2 = 0.53), and %N (R^2 = 0.57), all of which are multicolinear ($0.71 < r < 0.90$). ANaR was most strongly correlated with the ORG × %N, i.e., with particulate organic nitrogen (PON) and the meiofaunal biomass (Table 4). Moreover, the effect of meiofauna varied significantly with PON. Figure 4 shows the plot of the measured data versus curves predicted by the multiple regression model at PON levels of 0.65, 3.25, and 6.5 g·m^{-2}. These levels correspond to the PON levels in NO_2Try, NO_2Fis2 + NO_2Fis3, and NO_2Fis4, respectively (Table 1).

Discussion

Meiofaunal composition and abundance

The meiofauna in the heterotrophic microcosms and sand filters is very different from the meiofauna occurring in natural marine sands or freshwater trickling filters. It is copepod dominated whereas marine sand meiofauna is nematode dominated (Higgins and Thiel 1988) and freshwater trickling filter meiofauna is fly larvae and rotifer dominated (Andersson et al. 1994; Lee and Welander 1994).

The meiofauna of both the microcosms and sand filters is mainly composed of prehensile epibenthic species, such as the harpacticoids *Pseudonychocamptus*, or strongly adhesive species, such as the nematode *Anticoma*. There are only a few rare mesopsammic species (e.g., *Paramphisciella*). The fact that the sand filters are regularly backwashed may prevent the installation of mesopsammic species. Also, the fact that water is forced through the sand in both the microcosms and the sand filters could favor epibenthic species at the expense of mesopsammic ones. Hence the observed paucity of nematodes and rotifers, which are known to reduce nitrification in freshwater trickling filters (Lee and Welander 1994).

Most of the meiofaunal species found here generally eat diatoms, microalgae, or bacteria. *Anticoma*, for example, is a known selective deposit feeder (Wieser 1959). *Pseudonychocamptus* feeds not only on microalgae and diatoms (Rudnick 1989) but also on bacterial exopolymer (Decho and Moriarty 1990). *Tisbe furcata* has a clear preference for bacteria (Vanden Berghe and Bergmans 1981), and

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Fig. 3. Regression models between ANaR and meiofaunal biomass for the (*a*) tryptone experiment and (*b–d*) fishmeal experiments 2, 3, and 4.

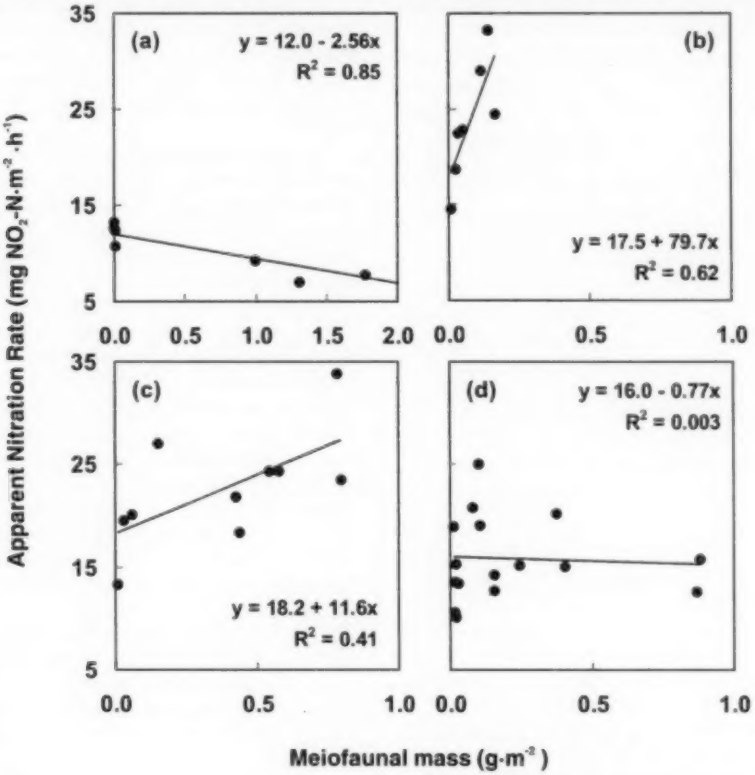

Table 2. Pearson correlations between ANR and ciliate density or meiofaunal biomass.

	Ciliates		Meiofaunal biomass		
Experiment	*r*	*p*	*r*	*p*	df
Nitrition					
NH_3Try	–0.15	0.72	–0.35	0.40	6
NH_3Det	–0.10	0.81	0.01	0.98	6
Nitration					
NO_2Try	0.01	0.98	–0.89	0.003	6
NO_2Fis2	0.27	0.56	0.81	0.03	5
NO_2Fis3	0.39	0.27	0.64	0.05	8
NO_2Fis4	0.20	0.45	0.20	0.46	14
Subsets of NO_2Fis3					
Biomass ≤0.16 g·m^{-2}	0.26	0.74	0.94	0.05	2
Biomass >0.16 g·m^{-2}	0.19	0.72	0.70	0.12	4
Subsets of NO_2Fis4					
Biomass ≤0.12 g·m^{-2}	0.28	0.48	0.77	0.02	7
Biomass >0.20 g·m^{-2}	0.74	0.15	–0.46	0.44	3

Paramphiascella for specific strains of Gram-negative bacteria (Rieper 1982). In our experiments, most meiofaunal species had to feed on bacteria, since the microcosms were kept in the dark and were devoid of algae.

Conditions of the nitrification experiments

Nitrate levels were higher in the tryptone nitration experiment than in the three fishmeal experiments (Table 1). The effect of this difference is unknown, but Spotte (1991) indicated that nitration is not affected by nitrate. He stated that nitrification is normal in seawater with 200 mg NO_3-N·L^{-1} or more.

The tryptone experiment also differed from the three fishmeal experiments in the type and amount of organic matter, suggesting that the biomass of nitrate bacteria was either lower or less active than in the fishmeal experiments. Tryptone is dissolved organic matter whereas fishmeal is particulate organic matter. The organic matter content of the sand in the tryptone experiment was only 50% of that found in the fishmeal experiments (Table 1). Although nitration in the tryptone experiment was stimulated before NO_2Try, nitration in the absence of meiofauna (as indicated by the intercept of the regressions) was slower than in the three fishmeal experiments (Fig. 2).

The correlation tests between ciliates and ANR showed that protozoan densities could not account for the observed variability in ANiR and ANaR in our nitrification experiments. Protozoans, especially ciliates, are known to affect nitrification (Johannes 1965; Pomeroy 1970; Barsdate et al. 1974), but either they equally affected all microcosms or their effect was too small compared with that of meiofauna in the microcosms.

Regression models of the nitration experiments

The results of the nitration experiments showed that the variability in ANaR is best explained by the meiofaunal bio-

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Table 3. Linear regression models and submodels of the nitrification experiments.

Experiment	Model	n	F	p	R^2	95% confidence interval of the slope
Nitrition						
NH_3Try	ANiR = 14.0 – 3.8 × biomass	8	0.82	0.40	0.12	$-14.0 < \beta_1 < 6.44$
NH_3Det	ANiR = 79.2 + 0.15 × biomass	8	6.65	0.05	0.0002	$-11.7 < \beta_1 < 12.0$
Nitration						
NO_2Try	ANaR = 12.0 – 2.56 × biomass	8	32.7	0.001	0.85	$-3.66 < \beta_1 < -1.47$
NO_2Fis2	ANaR = 17.5 + 79.7 × biomass	7	8.2	0.03	0.62	$8.03 < \beta_1 < 151.3$
NO_2Fis3	ANaR = 18.2 + 11.6 × biomass	10	5.5	0.05	0.41	$0.15 < \beta_1 < 23.0$
NO_2Fis3, ≤0.16 g·m^{-2}	ANaR = 14.7 + 84.0 × biomass	4	16.8	0.05	0.89	$0.05 < \beta_1 < 172.2$
NO_2Fis3, >0.16 g·m^{-2}	ANaR = 10.9 + 22.6 × biomass	6	4.1	0.11	0.51	$-8.35 < \beta_1 < 153.4$
NO_2Fis4	ANaR = 16.0 – 0.77 × biomass	16	0.04	0.84	0.003	$-8.87 < \beta_1 < 7.33$
NO_2Fis4, ≤0.12 g·m^{-2}	ANaR = 12.0 + 97.4 × biomass	9	10.1	0.02	0.59	$24.8 < \beta_1 < 169.9$
NO_2Fis4, ≥0.20 g·m^{-2}	ANaR = 18.1 – 4.24 × biomass	5	0.54	0.52	0.21	$-49.6 < \beta_1 < 31.0$

Table 4. Details of the multiple regression models of the square root of ANaR (mg$^{0.5}$ NO_2-N·m^{-1}·h$^{-0.5}$) over ORG (g·m^{-2}), its %N, PON (=ORG × %N, g·m^{-2}), and meiofaunal biomass (SQMEIO, g$^{0.5}$·m^{-1}) (n = 41).

	Effect			
	Coefficient	Partial R^2	SE	Two-tailed p
Model with ORG (R^2 = 0.534, RMS = 0.307, F ratio = 44.645, p = 0.000)				
Constant	2.053		0.322	0.000
ORG	0.010		0.001	0.000
Model with %N (R^2 = 0.668, RMS = 0.259, F ratio = 24.81, p = 0.000)				
Constant	3.350		0.290	0.000
%N	36.196	0.040	17.122	0.041
SQMEIO	–0.964	0.074	0.361	0.011
SQMEIO × %N	90.147	0.103	26.600	0.002
Model with PON (R^2 = 0.674, RMS = 0.226, F ratio = 25.52, p = 0.000)				
Constant	3.462		0.183	0.000
PON	0.111	0.040	0.051	0.036
SQMEIO	–0.527	0.042	0.185	0.007
SQMEIO × PON	0.274	0.089	0.076	0.001

mass and the PON content of the sediment. There is a relationship between ANaR and meiofaunal biomass, but it can be masked under certain conditions. Depending on the PON content of the sediment, this relationship is either negative or positive. At PON loads larger than 3.25 g·m^{-2}, meiofaunal masses less than 1.0 g·m^{-2} can increase nitration by up to fivefold.

A simple way to explain the variability of the effect of meiofauna on ANaR is to say, as for mineralizing bacteria (Meyer-Reil and Faubel 1980; Yingst and Rhoads 1980), that low meiofaunal masses stimulate nitrate bacteria by removing senescent cells and by keeping colonies in exponential growth whereas high masses reduce their number through grazing. The y-intercepts of the regression models in Table 3 give an indication of the nitrate bacteria abundance. When autotrophic density is low, as in NO_2Try, meiofauna has a negative effect, and when autotrophic density is high, as in NO_2Fis2 and NO_2Fis3, meiofauna has a positive effect. Also, this would explain why, within a given experiment such as NO_2Fis3, the relationship between meiofauna and ANaR varies with meiofaunal mass. This cannot be the whole explanation, however. First, autotrophic metabolism is at least 24 times slower than heterotrophic metabolism (Lee and Welander 1994). Second, heterotrophic bacteria are 20–100 times more abundant than nitrifying bacteria in the filterbed of a seawater aquarium (Yoshida 1967). And third, heterotrophic bacteria are more palatable and easier to graze than nitrifiers (Griffiths 1989). We thus believe that the simple explanation for the relationship between meiofauna and nitrating bacteria given above is not complete. We agree with Lee and Welander (1994) that, in fact, the relationship between meiofauna and ANaR is far more complex than it seems.

The meiofauna in our experiments was mainly composed of harpacticoid copepods (98% of the biomass). We know from our mineralization experiments in the same heterotrophic microcosms that this meiofauna feeds heavily on mineralizing bacteria: 1 g meiofauna·m^{-2} may reduce mineralization by up to 42% (Parent and Morin 1999). Perlmutter and Meyer (1991) have shown that stream-dwelling harpacticoid copepods reduce the biomass of detritally associated bacteria (i.e., heterotrophs) by as much as 45%. We also know that harpacticoid copepods are selective feeders. They are specialists with respect to nutritional requirements, nutritional preferences, and ability to utilize specific food species. Rieper (1982) has shown that four common harpacticoid genera do prefer Gram-negative bacteria and that each genus has its own food preference, e.g., *Vibrio* sp. for *Tisbe* and *Flavobacterium* sp. for *Paramphiascella*. Vanden Berghe and Bergmans (1981) found that *Tisbe*

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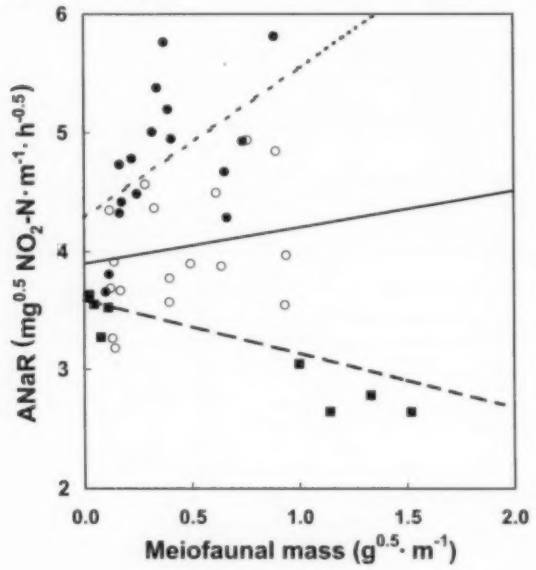
Fig. 4. Plot of all nitration results grouped into PON levels and of the corresponding PON curves (0.65, 3.25, and 6.5 $g \cdot m^{-2}$) predicted by the multiple regression of the square root of ANaR over PON and the square root of meiofaunal biomass (see Table 4 for details). PON levels ($g \cdot m^{-2}$): 0.5–0.8 (■), 1.8–4.6 (○), and 5.2–7.8 (●).



furcata had a clear preference for rod-shaped bacteria. More recently, Perlmutter and Meyer (1991) have shown that stream-dwelling *Attheyella* selectively removed larger rod-shaped bacteria. Unfortunately, nitrifying bacteria were not included in these food selectiveness experiments, nor were they studied in the field. We suspect that harpacticoid copepods have no clear preference for nitrifiers because these grow in large agglomerates (also known as zoogleas), which are more difficult to ingest than single cells. Moreover, nitrifiers are not surrounded by a mixture of enzymes and labile organic detritus that makes mineralizing bacteria so palatable to harpacticoids such as *Pseudonychocamptus* (Decho and Moriarty 1990).

Moreover, heterotrophic and autotrophic bacteria compete for space (Spotte 1991) and oxygen (Andersson et al. 1994) as well as for ammonium: for limiting amounts of ammonium, the numbers of nitrifying bacteria decreased with increasing glucose concentrations and the numbers of heterotrophic bacteria rose (Verhagen and Laanbroek 1992). Since harpacticoids are known to eat an important fraction of the biomass of mineralizing bacteria and since these are known to compete strongly with autotrophs, meiofauna may have indirectly influenced the ANaR of our experiments by removing heterotrophic bacteria. Meiofauna may therefore exert two opposite effects: a direct negative action by predation on nitrifiers and an indirect positive action by reducing competitors as they graze on heterotrophic bacteria.

Because bacterial masses were not measured, we do not know which effect was more important. However, we suspect that the reduction of competitors may be the main factor by which meiofauna had an effect on ANaR in the nitration experiments, due to the observed asymmetry in the effect of meiofauna (–20%·g meiofauna^{-1} in the tryptone experiment versus +172 to +571% in the fishmeal experiments) and to the absence in our meiofauna of most of the known grazers of nitrifiers, such as filter fly larvae (Psychodidae), snails (*Physa* spp.), worms (Naididae), rotifers (*Philodina* spp.), and nematodes (*Diplogaster* spp.) (Boller and Gujer 1986; Parker et al. 1989; Andersson et al. 1994; Lee and Welander 1994). All of these groups live in freshwater and were found in trickling filters consuming the biofilm on the trickling media. A second reason is that species of rotifers and nematodes, present in our meiofauna, had very low densities. Moreover, these species apparently do not feed on nitrifiers (Lee and Welander 1994). We found no mention of harpacticoid copepods selectively feeding on nitrifiers.

We therefore suggest the following explanation for the variability of meiofaunal effect on nitrate bacteria with the PON content of the sediment, assuming that the *y*-intercepts of the regression models (Table 3) and the PON levels (Table 1) are good indications of autotroph and heterotroph abundances, respectively. When organic matter is low, as in NO_2Try, heterotrophs are not abundant. Even low densities of harpacticoids need to feed not only on heterotrophs but also on autotrophs to sustain themselves, resulting in a negative effect of meiofauna on nitration. Conversely, when organic matter is abundant, as in NO_2Fis2 and NO_2Fis3, heterotrophs are also abundant. Low densities of harpacticoids need to feed only on heterotrophs to sustain themselves, resulting in an increase in the number of autotrophs and in a strong positive effect of meiofauna on nitration. High densities of harpacticoids will also have to feed on autotrophs, thus reducing the abundance of autotrophs as well as its positive effect on ANaR.

NO_2Fis4 is found in between these two situations. At low biomass, meiofauna probably fed only on heterotrophs whereas at high biomass, meiofauna ate some autotrophs as well. There was a positive effect of the meiofauna on ANaR which occurred at lower biomasses than for NO_2Fis2 and NO_2Fis3 because of a lower PON content (Table 3; Fig. 4). It was probably followed by a negative effect of meiofauna that could not be observed directly but that resulted in an apparent noneffect of meiofauna on nitration.

The positive effect of meiofauna at medium and high PON levels in marine microcosms contradicts the findings in freshwater trickling filters where freshwater organisms always decreased or reduced to nil the ANaR of the system (Boller and Gujer 1986; Parker et al. 1989; Andersson et al. 1994; Lee and Welander 1994). Our findings suggest that the effect of meiofauna on nitration varies with the composition of the meiofauna, which is determined, among other things, by water type and substrate type.

Nitrition experiments

The nitrition experiments exhibited a wide range of $ANiR_0$: from 7 to 90 $mg \cdot m^{-2} \cdot h^{-1}$ (Fig. 2). Nowhere within that range has meiofauna had any effect on ANiR with biomasses of up to 1.84 $g \cdot m^{-2}$. Contrary to nitration, nitrition is a situation where heterotrophs strongly compete with nitrifiers for ammonium (Verhagen and Laanbroek 1992). If meiofauna was feeding only on heterotrophs, we

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would probably have observed a positive effect of meiofauna on nitrition. Conversely, if meiofauna was feeding only on nitrifiers, we would probably have observed a negative effect of meiofauna on nitrition. We therefore hypothetize that, as for the nitration experiments, meiofauna exerts a dual action on nitrition.

When at low masses, meiofauna would graze only on heterotrophs; the decrease in ammonium intake by heterotrophs would be counterbalanced by an increase in ammonium intake by nitrifiers, through an increase either in biomass or in metabolic activity. There would be an increase in nitrition, but it would be masked by a decrease in assimilation by heterotrophs, resulting in no effect of meiofauna on ANiR, as observed here.

At high masses, meiofauna would graze on both heterotrophs and nitrifiers, and we would expect a negative effect to show up. Our meiofaunal masses were probably not large enough (less than 2.0 $g \cdot m^{-2}$) to show a significant negative effect as in the nitration experiments. The intake of ammonium by heterotrophs probably masks this negative effect over a wide range of meiofaunal masses. It would therefore appear that, as for nitration, the results are best explained by a dual action of meiofauna on bacteria.

Copepod-dominated meiofauna therefore has no apparent effect on nitrition at biomass levels less than 1.8 $g \cdot m^{-2}$, but, through nitration, it has a variable effect on the nitrification rate in heterotrophic marine habitats. At low PON levels, meiofaunal biomasses decrease nitrification, and at high PON levels, biomasses less than 0.16 $g \cdot m^{-2}$ increase nitrification two to five times. These results suggest that copepod-dominated meiofauna exerts a direct negative action by predation on nitrifiers and an indirect positive action by reducing competitors as they graze on heterotrophic bacteria.

Acknowledgments

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References

Alkemade, R., Wielemaker, A., de Jong, S.A., and Sandee, A.J.J. 1992. Experimental evidence for the role of bioturbation by the marine nematode *Diplolaimella dievengatensis* in stimulating the mineralization of *Spartina angli*. Mar. Ecol. Prog. Ser. **90**: 149–155.

American Public Health Association (APHA), American Water Works Association, and Water Pollution Control Federation. 1992. Standard methods for the examination of water and wastewater. 18th ed. American Public Health Association, Washington, D.C. pp. 4–91.

Andersson, B., Aspegren, H., Parker, D.S., and Lutz, M.P. 1994. High rate nitrifying trickling filters. Water Sci. Technol. **29**: 47–52.

Barsdate, R.J., Fenchel, T., and Prentki, R.T. 1974. Phosphorus cycle of model ecosystems: significance for decomposer food chains and effects of bacterial grazers. Oikos, **25**: 239–251.

Boller, M., and Gujer, W. 1986. Nitrification in tertiary trickling filters followed by deep-bed filters. Water Res. **20**: 1363–1373.

Curds, C.R. 1975. Protozoa. The organisms and their ecology. *In* Ecological aspects of used water treatment. Vol. 1. *Edited by* C.R. Curds and H.A. Hawkes. Academic Press, London, U.K. pp. 203–268.

DeAngelis, D.L. 1992. Dynamics of nutrient cycling and food webs. Chapman and Hall, London, U.K.

Decho, A.W., and Moriarty, D.J.W. 1990. Bacterial exopolymer utilization by a harpacticoid copepod: a methodology and results. Limnol. Oceanogr. **35**: 1039–1049.

Difco. 1984. Difco manual. Dehydrated culture media and reagents for microbiology. 10th ed. Difco Laboratories, Detroit, Mich. p. 1027.

Feller, R.J., and Warwick, R.M. 1988. Energetics. *In* Introduction to the study of meiofauna. *Edited by* R.P. Higgins and H. Thiel. Smithsonian Institution Press, Washington, D.C. pp. 181–196.

Fenchel, T. 1967. The ecology of marine microbenthos. I. The quantitative importance of ciliates as compared with metazoans in various types of sediments. Ophelia, **4**: 121–137.

Fenchel, T. 1978. The ecology of micro- and meiobenthos. Annu. Rev. Ecol. Syst. **9**: 99–121.

Findlay, S.E.G. 1981. Small scale spatial distribution of meiofauna on a mud- and sandflat. Estuarine Coastal Shelf Sci. **7**: 255–268.

Findlay, S., and Tenore, K.R. 1982. Effect of a free-living marine nematode (*Diplolaimella chitwoodi*) on detrital carbon mineralization. Mar. Ecol. Prog. Ser. **8**: 161–166.

Griffiths, B.S. 1989. The effect of protozoan grazing on nitrification — implications from the application of organic wastes applied to soils. *In* Nitrogen in organic wastes applied to soils. *Edited by* J.A.A. Hansen and K. Henriksen. Academic Press, London, U.K. pp. 37–46.

Higgins, R.P., and Thiel, H. 1988. Introduction to the study of meiofauna. Smithsonian Institution Press, Washington D.C.

Johannes, R.E. 1965. Influence of marine Protozoa on nutrient regeneration. Limnol. Oceanogr. **10**: 434–442.

Lee, N.M., and Welander, T. 1994. Influence of predators on nitrification in aerobic biofilm processes. Water Sci. Technol. **29**: 355–363.

Meyer-Reil, L.-A., and Faubel, A. 1980. Uptake of organic matter by meiofauna organisms and interrelationships with bacteria. Mar. Ecol. Prog. Ser. **3**: 251–256.

Parent, S., and Morin, A. 1999. The role of copepod-dominated meiofauna in the mineralization of organic matter in a cold marine mesocosm. Can. J. Fish. Aquat. Sci. **56**. In press.

Parker, D., Lutz, M., Dahl, R., and Bernkopf, S. 1989. Enhancing reaction rates in nitrifying trickling filters through biofilm control. J. Water Pollut. Control Fed. **61**: 618–631.

Perlmutter, D.G., and Meyer, J.L. 1991. The impact of a stream-dwelling harpacticoid copepod upon detritally associated bacteria. Ecology, **72**:2170–2180.

Pfannkuche, O., and Thiel, H. 1988. 9. Sample processing. *In* Introduction to the study of meiofauna. *Edited by* R.P. Higgins and H. Thiel. Smithsonian Institution Press, Washington, D.C. pp. 134–145.

Pomeroy, L.R. 1970. The strategy of mineral cycling. Annu. Rev. Ecol. Syst. **1**: 171–190.

Rieper, M. 1982. Feeding preferences of marine harpacticoid copepods for various species of bacteria. Mar. Ecol. Prog. Ser. **7**: 303–307.

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Rudnick, D.T. 1989. Time lags between the deposition and meiobenthic assimilation of phytodetritus. Mar. Ecol. Prog. Ser. **50**: 231–240.

Spotte, S. 1991. Captive seawater fishes. Science and technology. John Wiley & Sons, Toronto, Ont.

Stoskopf, M.K. 1993. Fish medicine. W.B. Saunders, Montreal, Que.

Sudo, R., and Aiba, S. 1984. Role and function of Protozoa in the biological treatment of polluted waters. *In* Advances in Biochemical Engineering/Biotechnology. *Edited by* A. Fiechter. Springer-Verlag, Berlin. pp. 117–141.

Tenore, K.R., Tietjen, J.H., and Lee, J.J. 1977. Effect of meiofauna on incorporation of aged eelgrass, *Zostera marina*, detritus by the polychaete *Nephthys incisa*. J. Fish. Res. Board Can. **34**: 563–567.

Vanden Berghe, W., and Bergmans, M. 1981. Differential food preferences in three co-occurring species of *Tisbe* (Copepoda, Harpacticoida). Mar. Ecol. Prog. Ser. **4**: 213–219.

Verhagen, F.J.M., and Laanboek, H.J. 1992. Effects of grazing by flagellates on competition between nitrifying and heterotrophic bacteria in chemostats. Appl. Environ. Microbiol. **58**: 1962–1969.

Watson, S.W., Bock, E., Harms, H., Koops, H.-P., and Hooper, A.B. 1989. *In* Bergey's manual of systematic microbiology. Vol. 3. Nitrifying bacteria. *Edited by* J.G. Holt. Williams & Wilkins, Baltimore, Md. pp. 1808–1834.

Wieser, W. 1959. Free-living marine nematodes. IV. General part. Acta Univ. Lund. N.F. Adv. 2, **55**: 1–111.

Yingst, J.Y., and Rhoads, D.C. 1980. The role of bioturbation in the enhancement of bacterial growth in marine sediments. *In* Marine benthic dynamics. *Edited by* K.R. Tenore and B.C. Coull. Belle W. Baruch Libr. Mar. Sci. **11**: 407–421.

Yoshida, Y. 1967. Studies on the marine nitrifying bacteria, with special reference to characteristics and nitrite formation of marine nitrite formers. Bull. Misaki Mar. Biol. Inst. Kyoto Univ. **11**: 1–58.

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Temperature-related loss of smolt characteristics in Atlantic salmon (*Salmo salar*) in the wild

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Abstract: Atlantic salmon (*Salmo salar*) that had previously been released as fry in tributaries of the Connecticut River were captured from 1993 to 1997 during their normal spring smolt migration 198 km from the mouth of the river. Smolts had peak levels of gill Na^+,K^+-ATPase activity and salinity tolerance early in migration (early May), indicating physiological readiness to enter seawater. Significant decreases in gill Na^+,K^+-ATPase activity (29–66%) and salinity tolerance were seen in smolts at the end of the migratory period (late May and early June). Reduced gill Na^+,K^+-ATPase activity occurred earlier in warm years and was directly related to the degree-days during migration (r^2 = 0.75). Reduced gill Na^+,K^+-ATPase activity was found at the end of migration in warmer, southern rivers (Connecticut River and Penobscot River, Maine) but not in northern rivers (Catamaran Brook, New Brunswick, and Conne River, Newfoundland). Both hatchery- and stream-reared fish held in the laboratory exhibited a more rapid loss of physiological smolt characteristics when held at higher temperature. The results indicate that late migrants in southern rivers lose physiological smolt characteristics due to high temperatures during spring migration. Delays in migration, such as those that occur at dams, may have negative impacts on smolt survival in warmer rivers.

Résumé : Les saumons atlantiques (*Salmo salar*), libérés à l'état d'alevin dans les affluents du fleuve Connecticut, ont été capturés entre 1993 et 1997, pendant leur migration normale au printemps à l'état de smolt, à 198 km de l'embouchure du fleuve. Au début de la migration (début mai), l'activité Na^+,K^+-ATPasique et la tolérance à la salinité atteignaient un pic, ce qui est une indication qu'ils sont prêts à pénétrer en eau salée. Des baisses importantes de l'activité Na^+,K^+-ATPasique (29–66%) et de la tolérance à la salinité ont été observées chez les smolts à la fin de la période de migration (fin mai et début juin). Une réduction de l'activité Na^+,K^+-ATPasique a été observée plus tôt les années chaudes, et elle était directement liée aux degrés-jours pendant la migration (r^2 = 0,75). Cette réduction a été constatée à la fin de la migration dans les eaux chaudes des cours d'eau du sud (fleuve Connecticut et rivière Penobscot, dans le Maine), mais pas dans les rivières du nord (ruisseau Catamaran, au Nouveau-Brunswick, et rivière Conne, à Terre-Neuve). Les poissons d'élevage et les poissons sauvages maintenus au laboratoire perdaient plus rapidement les caractéristiques physiologiques des smolts lorsqu'ils étaient exposés à une température plus élevée. Les résultats montrent que les migrants tardifs des rivières du sud perdent les caractéristiques physiologiques du smolt à cause de la température élevée pendant la migration printanière. Les retards dans la migration, comme ceux attribuables aux barrages, peuvent avoir des effets négatifs sur la survie des smolts dans les cours d'eaux chaudes.

[Traduit par la Rédaction]

Introduction

At the time of downstream migration in spring, smolts develop salinity tolerance and undergo other physiological and behavioral changes in preparation for seawater entry. Increased salinity tolerance is the result of changes in the gill, gut, and kidney, including higher gill Na^+,K^+-ATPase activity that provides ionic and electrical gradients used for Na^+ and Cl^- transport. These developmental changes are reversible; when prevented from entering seawater, hatchery-reared smolts lose their migratory urge, salinity tolerance, and the underlying osmoregulatory changes (Duston et al. 1991; see Hoar 1988 for review). Fish that have lost their smolt characteristics and are subsequently released have substantially reduced adult return rates (Virtanen et al. 1991; Staurnes et al. 1993). It is not currently known, however, whether loss of physiological smolt characteristics occurs in migrating salmon in the wild. Loss of these characteristics

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may be particularly important when migration is delayed, as might occur at dams and impoundments. Salmon inhabiting rivers with higher spring temperatures, such as those in the southern portion of their natural distribution, may have a greater risk of losing smolt characteristics because higher temperatures result in a more rapid loss of salinity tolerance and gill Na^+,K^+-ATPase activity in smolting salmonids (Adams et al. 1973; Zaugg and McLain 1976; Zaugg 1981; Duston and Saunders 1990).

Southern New England is historically the geographical southern limit of the distribution of Atlantic salmon (*Salmo salar*) in North America. Atlantic salmon in the Connecticut and several other large New England rivers were extirpated in the early 1800s following dam construction (Moffitt et al. 1982). A restoration effort begun in 1967 originally involved release of smolts from a variety of North American stocks (Rideout and Stolte 1988). A more recent strategy for restoration of Atlantic salmon to rivers of New England involves hatchery spawning of returning adults and release of progeny into tributaries as recently hatched fry (McMenemy 1995; Orciari and Leonard 1996). Although adult returns to the Connecticut river have been relatively stable, returns to other New England rivers have experienced substantial declines in the last decade.

The present study was undertaken to determine whether loss of salinity tolerance occurs in Atlantic salmon in nature and to examine the potential environmental factors involved. McCormick and Björnsson (1994) made physiological comparisons between wild- and hatchery-reared fish in the Connecticut River; however, they examined migrating fish at only a single time point and therefore could not resolve any differences that might occur over the migratory period. In the present study, we examine physiological changes in migrating smolts over the entire migratory period from several rivers with different spring temperature regimes. We hypothesized that if temperature-related loss of smolt characteristics occurs in nature, they would be detected in warmer, southern rivers and to a lesser extent or not at all in cooler, northern rivers. We also hypothesized that year-to-year variation in spring temperatures would affect the loss of smolt characteristics in the wild. Finally, stream-reared smolts were subjected to controlled temperature experiments in order to verify the results of earlier studies with hatchery-reared smolts, which demonstrated a temperature-related loss of smolt characteristics (Duston et al. 1991).

Materials and methods

Sampling sites

The Connecticut River is the largest river in New England, draining a 40 000-km^2 watershed and emptying into Long Island Sound. For the last 25 years, a restoration program released hatchery-produced smolts that originated primarily from the Penobscot River (Maine) but also included several other rivers (Rideout and Stolte 1988). Since 1987, more than a million fry have been released annually into tributaries of the Connecticut River. Progeny of Connecticut River returns comprise the bulk of current releases, but introductions from outside stocks (primarily Penobscot River) continued until 1995. Atlantic salmon released as fry reside in the streams for 2–3 years prior to smolt migration. From 1993 to 1997, these stream-reared fish were captured as smolts at a bypass facility at Cabot Station (a dam on the Connecticut River at Turners Falls, Mass., 198 km from the river mouth; Fig. 1). This facility was designed to facilitate movement of fish around the dam and contained a dewatering screen and water table for immediate capture and sampling of fish.

In 1995, smolts on the Penobscot River (drainage area = 22 000 km^2) were sampled at a bypass facility at Weldon Dam, 104 km from the river mouth. In 1995, smolts from Catamaran Brook, New Brunswick, were sampled at a fish counting fence located 0.2 km from the confluence with the Little Southwest Miramichi and 95 km from the estuary. Catamaran Brook (drainage area = 52 km^2) is a third-order tributary of the Miramichi River system and the site of a long-term salmon research project. Atlantic salmon are the most common species; smolts typically emigrate at age 3 and annual numbers range between 500 and 2500 (see Cunjak and Therrien 1997 for details). Due to high water, sampling on the Penobscot River and Catamaran Brook in other years was possible only for a small portion of the migratory period. From 1995 to 1997, smolts were captured from the Conne River, located on the south coast of Newfoundland, at a counting fence 12 km from the river mouth (see Dempson and Stansbury 1991 for details). Conne River has a drainage area of 602 km^2, and over the period 1987–1997 the annual production of wild smolts ranged from 55 000 to 100 000 (Dempson et al. 1998).

Migratory smolt sampling

In each of the rivers, sampling of smolts began as soon as at least 10 fish per day had migrated (less than 1% of the smolt run had passed before this date); sampling occurred weekly thereafter until migration ceased. On the Connecticut River, fish were sampled as soon as they appeared in the water table (maximum time of 15 min since entering the bypass). In the Penobscot River, Catamaran Brook, and Conne River, fish were removed from the capture box each morning. Routinely, 10–12 fish selected at random were sampled immediately after capture as described below. In some years at the end of the migratory period (June), fish numbers decreased and fewer than 10 fish were sampled at a given time point.

In addition to immediate sampling of smolts, 80 smolts from the Connecticut River were captured at the first sampling period in 1993 and 1994 and brought to the Conte Anadromous Fish Research Center (Turners Falls) so that physiological changes under known temperature conditions could be monitored. Fish were held in 1.8-m-diameter tanks supplied with river water at about 4 $L \cdot min^{-1}$ with aeration and fed commercial salmon pellets twice a day. Lighting was by daylight through overhead windows supplemented by natural-spectrum fluorescent lights controlled by timers to give a normal seasonal increase in daylength. At weekly intervals, 10 fish were sampled for gill biopsy and 10 fish were subjected to a seawater challenge. In 1996, Atlantic salmon juveniles in a power canal on the Deerfield River were sampled by angling or seining from June 17 to 20. The Deerfield River is a major tributary of the Connecticut River and the power canal was located 13 km from Cabot Station. These fish were large enough and had the appearance of smolts but had apparently stopped migrating and taken up residence in the power canal. On June 26, 1996, Atlantic salmon parr were captured by electrofishing on the Sawmill River, Montague, Mass., 9.6 km from Cabot Station. These fish were used to determine gill Na^+,K^+-ATPase activity in parr for comparison with smolts that were losing physiological smolt characteristics.

Temperature studies

Hatchery- and stream-reared smolts from the Connecticut River were subjected to different controlled temperature regimes in order to verify previous studies on the effect of temperature on loss of smolt characteristics in hatchery fish from more northern regions. Fish were reared from eggs at the White River National Fish Hatchery in Bethel, Vermont. On May 20, 1996, smolts (14–20 cm) were transferred to the Conte Anadromous Fish Research Center. Fish were randomly divided into two 1-m-diameter tanks

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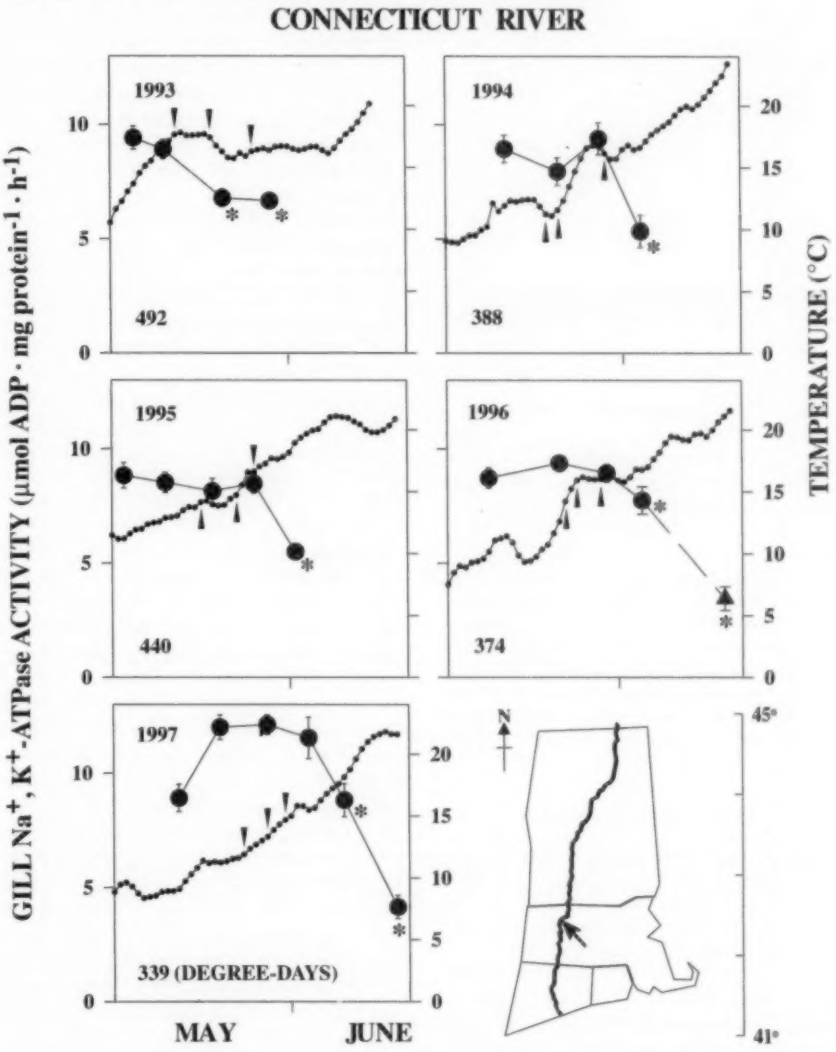
Fig. 1. Water temperature of the Connecticut river at Cabot Dam (arrow on map) and gill Na^+,K^+-ATPase activity (mean ± SE) in migrant, stream-reared Atlantic salmon smolts in 1993–1997 captured throughout the normal migratory period. Arrowheads on each temperature profile indicate the dates when 50, 75, and 95% of the total number of smolts have migrated (data from Northeast Utilities Service Company, Downstream Passage of Atlantic Salmon Smolts, 1993–1997). An asterisk indicates a significant difference from peak levels of gill Na^+,K^+-ATPase activity for that year (first or second sampling period; Kruskal–Wallis test, $P < 0.01$). In 1996, juvenile nonmigrant Atlantic salmon (17–21 cm fork length, triangle) were captured in a power canal on the Deerfield River, 13 km from Cabot Station. Degree-days in May for each year are shown in the lower left corner.



containing about 60 fish each and kept at 10–13°C for 3 days. Thereafter, one group was maintained on ambient river water (increasing from 11.0 to 20.0°C) and the second on chilled river water (11.0–13.0°C), each with a flow rate of 4 L·min^{-1}. Both groups were fed to satiation twice daily. Lighting was supplied by overhead natural-spectrum fluorescent lights and a simulated natural photoperiod was maintained by adjusting the on–off cycle twice a week. Feed was withheld for 24 h prior to sampling, which occurred at 10:00–11:00 EST. Fish were sampled monthly in the hatchery and every 10 days in the laboratory. On May 31, 1996, 10 fish from each group were subjected to a seawater challenge (35‰).

On May 27, 1997 (river temperature 13.9°C), 30 smolts were captured at Cabot Station and randomly divided into two groups. Both groups were held in 1.8-m-diameter tanks supplied with river water at about 4 L·min^{-1} with aeration and fed commercial salmon pellets twice a day. One group was supplied with river water at ambient temperature and the other with chilled river water (9.7–10.5°C). Two weeks after capture (June 11, 1997), fish were sampled for a gill biopsy and then subjected to a seawater challenge the following day.

Sampling methods

Fish were anesthetized (100 mg MS 222·L^{-1}, pH 7.0) and fork length and weight recorded. For gill Na^+,K^+-ATPase, a nonlethal biopsy was taken as described in McCormick (1993). In several experiments, blood was drawn from the caudal vessels into a 1-cm^3

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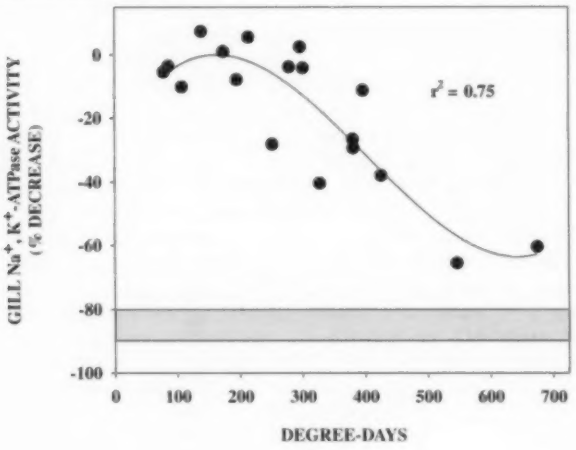
Fig. 2. Percent decrease in gill Na^+,K^+-ATPase activity as a function of degree-days in Atlantic salmon smolts migrating in the Connecticut River in 1993–1997. The change in gill Na^+,K^+-ATPase activity and degree-days was calculated from the peak levels of gill Na^+,K^+-ATPase activity for a given year (first or second sampling date).



heparinized syringe and spun at 3000 × *g* for 5 min at 4°C and plasma stored at –80°C until analysis.

Seawater challenge

Fish were transferred to 1-m-diameter tanks containing 40‰ seawater (artificial sea salt added to dechlorinated tap water) maintained at 10°C. Paper and charcoal filtration and continual aeration maintained low ammonia and high oxygen levels. After 24 h the surviving fish were anesthetized and sampled as described above. Plasma chloride and osmolality were used as indicators of the capacity to regulate ions following seawater exposure. In an effort to reduce mortality, 35‰ was used for seawater challenges in 1994 and subsequent years.

Gill Na^+,K^+-ATPase activity and plasma chloride

Four to six primary gill filaments were severed above the septum, placed in 100 µL of ice-cold SEI buffer (150 mM sucrose, 10 mM EDTA, 50 mM imidazole, pH 7.3), and frozen at –80°C within 30 min. Na^+,K^+-ATPase activity was measured by the method outlined in McCormick (1993). Plasma chloride was measured by coulometric titration (Labconco, Kansas City, Mo.).

Statistical analysis

Condition factor was calculated as (weight/length^3) × 100, with wet weight in grams and fork length in centimetres. Degree-days were calculated as the cumulative mean daily temperature. The statistical significance of changes over time in gill Na^+,K^+-ATPase activity and plasma ions in each of the stream-reared groups (migrant and captive) was examined by the nonparametric Kruskal–Wallis test; if significant differences occurred ($P < 0.05$), then nonparametric pairwise comparisons were made to the initial sampling date ($P < 0.01$). Comparisons of mortality in seawater challenge tests were performed using Fisher's exact test ($P < 0.05$). Influence of thermal regimes on gill Na^+,K^+-ATPase activity and plasma ions after seawater challenge was analyzed by two-way analysis of variance (ANOVA); if significant temperature effects were found ($P < 0.05$), a one-way ANOVA was used to compare groups at the same time point.

Results

Migrating smolts in the Connecticut River

From 1993 through 1997, Atlantic salmon smolts were captured during their normal migratory period at Cabot Station. Temperature differed substantially between years (Fig. 1). For example, the dates at which temperatures first reached 15°C were May 7, 1993, May 24, 1994, May 23, 1995, May 22, 1996, and May 30, 1997, and there was considerable variation in the number of degree-days.

Gill Na^+,K^+-ATPase activity was relatively high (8–10 µmol ADP·mg protein^{-1}·h^{-1}) at the first sampling period in all years (Fig. 1). The only year in which significant increases occurred after the first sampling was 1997, the coolest of the five years. In every year, a significant decrease in gill Na^+,K^+-ATPase activity occurred toward the end of the migratory period. In the warmest year (1993), significant decreases were found in May, whereas in all other years, significant decreases were not found until the very end of the migratory period in early June. In 1996, 17- to 21-cm fish that had the morphological appearance of smolts were captured in a power canal on the Deerfield River on June 17–20 (after the normal migratory period). These fish had a gill Na^+,K^+-ATPase activity of 3.5 ± 0.5 µmol ADP·mg protein^{-1}·h^{-1}, 63% lower then peak smolt levels (Fig. 1). Parr (9–13 cm) collected in the Sawmill River (a tributary of the Connecticut River) on June 26 had a gill Na^+,K^+-ATPase activity of 2.0 ± 0.2 µmol ADP·mg protein^{-1}·h^{-1}.

Degree-days from peak levels could explain 75% of the variation in percent change from peak levels of gill Na^+,K^+-ATPase activity over the five years of sampling (Fig. 2). Several independent variables were tested for their ability to predict changes in absolute and percent change in gill Na^+,K^+-ATPase activity from peak levels. Using best subsets and forward stepwise regression, degree-days from the peak levels was chosen over date, days from May 1, degree-days from May 1, temperature, and number of days over 15°C.

In 1993 and 1994, 10 fish at each sampling period were subjected to a seawater challenge. In both years, salinity tolerance was greatest at the beginning of migration (Tables 1 and 2). Significant decreases in salinity tolerance (increases in plasma chloride and mortality after seawater challenge) occurred in the latter part of the migratory period in both years; decreases were detected as early as May 22 in 1993 and May 27 in 1994. Significant decreases in gill Na^+,K^+-ATPase activity and salinity tolerance also occurred in captive fish that were maintained in ambient river water in both years.

In 1993 and 1994, smolts captured at the beginning of the migratory period were smaller than those later in the run (Tables 1 and 2), and condition factor remained constant throughout the migration in both years. Similar patterns were observed in 1995–1997 (data not shown). In 1993, plasma chloride of migrants was highest in early May and was slightly but significantly lower on May 10 and 28. In 1994, there was no significant difference in plasma chloride over the course of the migration.

Migrating smolts in other North American rivers

In 1995, wild migrating smolts were sampled during their

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Table 1. Physiological changes in migrant and captive stream-reared juvenile Atlantic salmon captured at a bypass of Cabot Station, Turners Falls, on the Connecticut River during normal migration in 1993.

	Group	May 5	May 10/14	May 20/22	May 28/June 1
Length (cm)	Migrant	15.6±0.3	15.9±0.3	17.5±0.3*	18.2±0.6*
	Captive	—	14.2±0.3*	15.4±0.6	15.7±0.5
Weight (g)	Migrant	33.4±1.8	36.2±2.7	48.2±2.8*	54.2±6.3*
	Captive	—	22.8±1.2*	29.8±3.8	32.1±3.5
Condition factor ($g \cdot cm^{-3} \times 100$)	Migrant	0.87±0.01	0.89±0.02	0.89±0.02	0.88±0.03
	Captive	—	0.80±0.01*	0.79±0.02*	0.81±0.03
Gill Na^+,K^+-ATPase ($\mu mol\ ADP \cdot mg\ protein^{-1} \cdot h^{-1}$)	Migrant	9.4±0.5	8.9±0.4	6.8±0.4*	6.7±0.7*
	Captive	—	8.7±0.2	3.6±0.3*	4.6±0.5*
Plasma chloride (mM)	Migrant	136±1	128±1*	133±4	129±7*
	Captive	—	136±3	134±2	124±6
	Migrant SW	166±7	159±7	180±11*	202±16*
	Captive SW	—	182±8*	189±7*	183±10*
Mortality (%)	Migrant SW	0	0	40	0
	Captive SW	—	0	30	20

Note: Captive fish were those initially captured on May 5 and maintained in flowing river water in 1.8-m-diameter tanks. Values are mean ± SE (n = 10–15 per group). SW fish were those subjected to a 24-h seawater challenge. An asterisk indicates a significant difference from the May 5 migrants (Kruskal–Wallis test, $P < 0.01$). The first date represents the migrant group and the second the captive group.

Table 2. Physiological changes in migrant and captive stream-reared juvenile Atlantic salmon captured at a bypass of Cabot Station, Turners Falls, on the Connecticut River during normal migration in 1994.

	Group	May 11	May 20	May 27	June 3
Length (cm)	Migrant	14.9± 0.6	17.4±0.6*	18.4±0.6*	16.8±0.5
	Captive	—	15.2±0.3	14.9±0.4	15.7±0.4
Weight (g)	Migrant	29.8±4.3	48.5±4.7*	54.8±4.5*	41.0±3.6
	Captive	—	29.1±1.9	27.0±3.0	36.0±3.4
Condition factor ($g \cdot cm^{-3} \times 100$)	Migrant	0.85±0.01	0.89±0.01	0.87±0.03	0.85±0.02
	Captive	—	0.82±0.02	0.79±0.03	0.92±0.05
Gill Na^+,K^+-ATPase ($\mu mol\ ADP \cdot mg\ protein^{-1} \cdot h^{-1}$)	Migrant	8.9±0.6	8.0±0.6	9.4±0.7	5.3±0.7*
	Captive	—	8.4±0.3	4.8±0.6*	3.5±0.5*
Plasma chloride (mM)	Migrant	127±1	128±2	123±2	126±2
	Captive	—	127±2	133±2*	127±1
	Migrant SW	133±1	135±4	150±3*	138±3
	Captive SW	—	135±5	150±6*	157±4*
Mortality (%)	Migrant SW	0	50	40	70
	Captive SW	—	0	0	20

Note: Captive fish were those initially captured on May 11 and maintained in flowing river water in 1.8-m-diameter tanks. Values are mean ± SE (n = 10 per group). SW fish were those subjected to a 24-h seawater challenge. An asterisk indicates a significant difference from the May 11 migrants (Kruskal–Wallis test, $P < 0.01$).

normal migratory period on the Penobscot River, Catamaran Brook, and Conne River. River temperatures and changes in gill Na^+,K^+-ATPase activity for these rivers and the Connecticut River in 1995 are shown in Fig. 3. Gill Na^+,K^+-ATPase activity in smolts from the Penobscot River, Catamaran Brook, and Conne River was initially lower than the levels seen in the Connecticut River. Smolts from the Penobscot River had increased gill Na^+,K^+-ATPase activity in the middle of the run and then subsequently had significantly lower levels in June. In contrast, gill Na^+,K^+-ATPase activity in smolts from Catamaran Brook and the Conne River increased steadily throughout the migratory period, with no decrease at the end of the migratory period. This pattern of low initial gill Na^+,K^+-ATPase activity with steadily increasing values throughout migration occurred in all three years of sampling on the Conne River (Fig. 4).

Temperature studies

Hatchery-reared fish were subjected to two temperature regimes (ambient and chilled) beginning on May 20, 1996 (Fig. 5). Gill Na^+,K^+-ATPase activity in hatchery-reared smolts on ambient river water decreased rapidly and significantly from peak levels of 10.0 $\mu mol\ ADP \cdot mg\ protein^{-1} \cdot h^{-1}$ to 4.9 $\mu mol\ ADP \cdot mg\ protein^{-1} \cdot h^{-1}$ after 10 days and to 2.0 $\mu mol\ ADP \cdot mg\ protein^{-1} \cdot h^{-1}$ after 22 days. In contrast, there was no significant change in gill Na^+,K^+-ATPase activity in smolts maintained on chilled river water (11–13°C) after 10 days and a significant decrease to 5.4 $\mu mol\ ADP \cdot mg\ protein^{-1} \cdot h^{-1}$ after 22 days. Gill Na^+,K^+-ATPase activity in fish on chilled river water was significantly greater than in fish on ambient river water at both sampling periods. Salinity tolerance on May 31, 1996, was significantly lower in fish on ambient river water (plasma chloride = 175 ±

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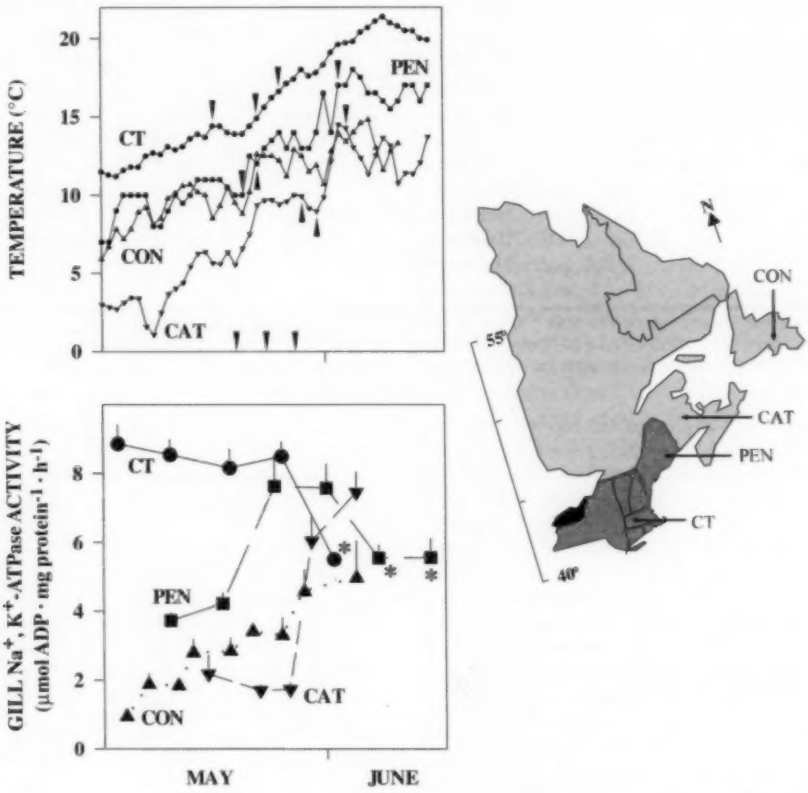
Fig. 3. Water temperature and gill Na^+,K^+-ATPase activity (mean ± SE) in migranting Atlantic salmon smolts in four rivers in North America in 1995. CT, Connecticut River (circles); PEN, Penobscot River (squares); CAT, Catamaran Brook (inverted triangles); CON, Conne River (righted triangles). Degree-days in May were 440 (CT), 340 (PEN), 187 (CAT), and 309 (CON). Arrowheads on each temperature profile indicate the dates when 50, 75, and 95% of the total number of smolts have migrated (Conne River on *x*-axis). An asterisk indicates a significant decrease from peak levels of gill Na^+,K^+-ATPase activity for a particular river (first or second sampling period; Kruskal–Wallis test, $P < 0.01$).



5.3 mM) than in fish on chilled river water (plasma chloride = 151 ± 2.1 mM).

Stream-reared fish captured at Cabot Station on May 27, 1997, were subjected to two temperature regimes, ambient river temperature (increasing from 13.9 to 19.2°C) and 10°C (9.7–10.5°C). Gill Na^+,K^+-ATPase activity upon capture was 12.1 ± 0.4 µmol ADP·mg protein^{-1}·h^{-1} (n = 12). After 14 days at ambient temperature (258 degree-days), gill Na^+,K^+-ATPase activity had decreased by 75% to 3.1 ± 0.3 µmol ADP·mg protein^{-1}·h^{-1} (n = 13). Gill Na^+,K^+-ATPase activity in smolts held at 10°C (148 degree-days) had decreased to 6.8 ± 1.0 µmol ADP·mg protein^{-1}·h^{-1} (n = 12) but was more than twofold higher than that in fish maintained at ambient temperature (Kruskal–Wallis test, $P < 0.001$). Plasma chloride after seawater challenge was significantly greater in smolts at ambient temperature (179 ± 4.9 mM) than in smolts at 10°C (158 ± 2.6 mM).

To examine whether holding the temperature of seawater challenges constant throughout the study while varying freshwater-rearing temperature affected measurement of salinity tolerance, two experiments were conducted with hatchery fish at seasonally varying temperatures in late winter (2°C) and late spring (16°C). Fish in freshwater at 2°C were transferred to 35‰ at 2 and 10°C; plasma chloride after 24 h was 168 ± 4 and 177 ± 2 mM, respectively (mean ± SE, n = 10 per group, Student t test, P = 0.06,). Fish in freshwater at 16°C were transferred to 35‰ at 16 and 10°C; plasma chloride after 24 h was 165 ± 4 and 158 ± 4 mM, respectively (n = 10 per group; Student t test, P = 0.19). In each case, isothermal seawater transfer was not significantly different from those at 10°C.

Discussion

Lower gill Na^+,K^+-ATPase activity and salinity tolerance were seen at the end of the migratory period in all years that fish were sampled in the Connecticut River. The first appearance of decreases and the degree of decrease were directly related to the temperature regime (Figs. 1 and 2). In the Penobscot River, which had relatively warm temperatures in late spring, smolts also had significantly lower gill Na^+,K^+-ATPase activity at the end of the run (Fig. 3). In contrast, the more northern Catamaran Brook and Conne River had no loss of gill Na^+,K^+-ATPase activity at the end of the migratory period. The pattern of increases seen in Catamaran Brook in 1995 and the Conne River in 1995–

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Fig. 4. Water temperature and gill Na^+,K^+-ATPase activity (mean + SE) in wild migrant Atlantic salmon smolts in the Conne River sampled in 1995 (circles), 1996 (squares), and 1997 (triangles). Degree-days in May were 309 (1995), 290 (1996), and 223 (1997). In the upper panel, symbols for each year at the bottom indicate the time of 50, 75, and 95% of the smolt migration.

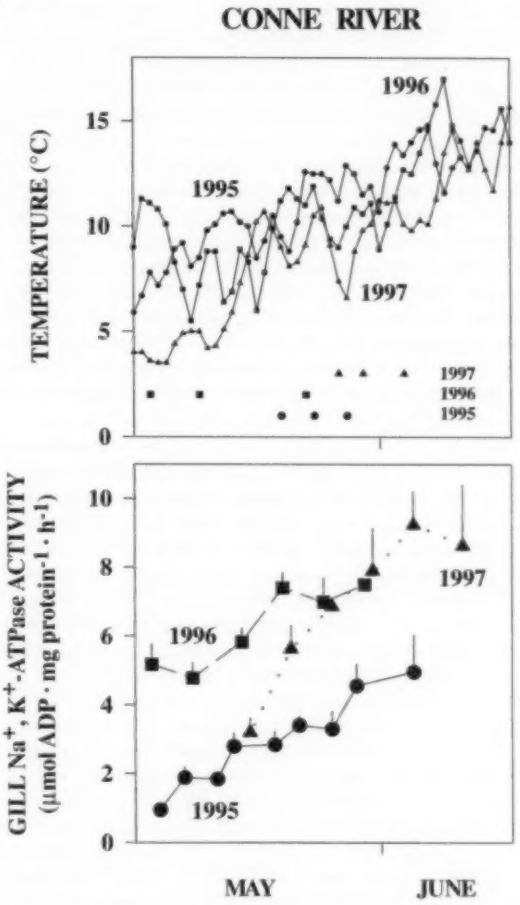
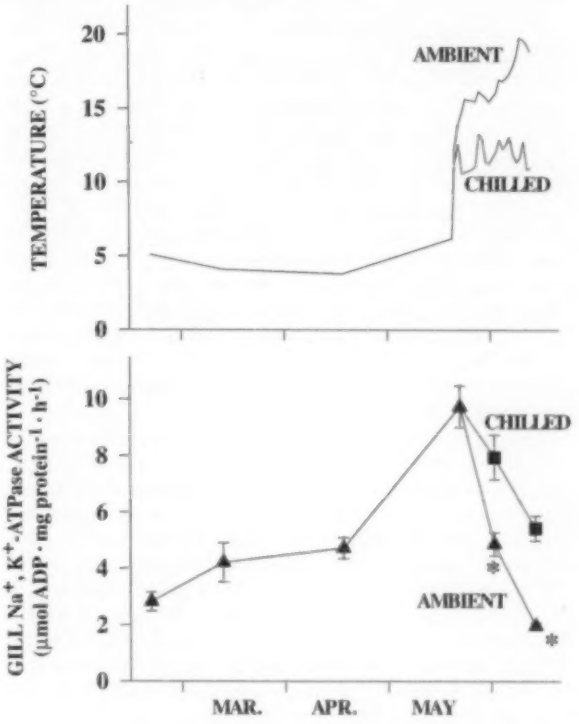


Fig. 5. Water temperature and gill Na^+,K^+-ATPase activity (mean ± SE, $n = 10$ per group) in Atlantic salmon reared at a hatchery and exposed to ambient (triangles) or chilled (sqaures) river water. Ambient river temperatures in May and June are nearly identical to those experienced by fish in the wild. An asterisk indicates a significant difference from the chilled group (Kruskal–Wallis test, $P < 0.01$).



1997 was very similar, with low levels initially followed by increases throughout the migratory period (Figs. 2 and 3).

A temperature-related loss of smolt characteristics is the most likely explanation for the observed patterns of physiological changes in wild and stream-reared Atlantic salmon smolts. Laboratory studies of hatchery- and stream-reared fish in the present study indicate that reducing temperature below ambient in spring reduces the rate of loss of physiological smolt characteristics. This finding agrees with previous research demonstrating that for several species of smolting salmonids the loss of salinity tolerance and other smolt characteristics in hatchery- or laboratory-reared fish occurs more rapidly at higher temperatures (Adams et al. 1973; Zaugg and McLain 1976; Zaugg 1981; Duston et al. 1991). That temperature is the primary determinant is demonstrated by the clear and direct relationship between degree-days (cumulative daily temperature) and loss of smolt characteristics under both constant and increasing temperature in captive populations (Duston et al. 1991; McCormick et al. 1997) and fish in the wild (Fig. 2). This relationship between temperature (degree-days) and loss of smolt characteristics can explain most of the observed patterns in loss of smolt characteristics in naturally migrating populations of Atlantic salmon in the Connecticut and Penobscot rivers.

There is substantial evidence that smolt survival and subsequent adult returns are dependent on the timing of smolt migration and the physiological preparedness of smolts. Release of hatchery-reared fish has shown sharp seasonal differences in adult return rates that are strongly correlated with smolt development, including very low return rates associated with loss of smolt physiology (Virtanen et al. 1991; Staurnes et al. 1993). The present study lends further support to the concept of a physiological "smolt window" in which there is a limited time for successful migration of smolts. This smolt window is controlled by environmental and biotic factors regulating the onset, development, and subsequent loss of salinity tolerance and other smolt characteristics (see Hoar 1988; McCormick et al. 1998). There is very limited information on the influence of run timing on smolt survival in wild populations. Hansen (1987) found that lake-reared smolts that migrated down the River Imsa (Norway) in late spring and throughout the summer had reduced

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survival compared with stream-reared fish that migrated primarily in May. In Catamaran Brook the average smolt-to-grilse return rate from 1990 to 1996 was 8.5% (Cunjak and Therrien 1997). The grilse return rate from the 1992 smolt run was only 4.6% and may have been due to the very late migration of many smolts (20% migrated after June 21) that was probably due to unusually low spring discharge. In the Conne River, years with later smolt migration subsequently have lower adult (grilse) return rates (Dempson et al. 1998). It should be noted that factors other than smolt physiology will also be important in determining changes in smolt survival during the normal migratory period. McCormick et al. (1998) suggested that there is both a physiological and an ecological smolt window that may have independent periods for high smolt survival. There may also be genetic factors that affect the interaction of temperature and loss of smolt characteristics. Loss of smolt characteristics has, however, been observed in hatchery populations derived from stocks from many latitudes in both Europe and North America (Whitesel 1993; McCormick et al. 1997). It is possible that the original stocks of the Connecticut River and elsewhere in New England were adapted to avoid these losses, through either early migration or resistance to temperature-induced loss of smolt physiology.

Loss of smolt characteristics in Atlantic salmon in the Connecticut River occurred after the peak of migration in all years. Nonetheless, in some years, a significant portion of the population will be affected by loss of smolt characteristics. The warmest spring of the years studied was in 1993, and decreased gill Na^+,K^+-ATPase activity was detected earlier (as both absolute date and time since the beginning of the migratory period) in 1993 than in any other year. Significant decreases in gill Na^+,K^+-ATPase activity were detected on May 20, 1993, and about 20% of the run occurred after this date. The fact that these changes were detected 198 km from the mouth of the Connecticut River indicates that the additional time needed to reach the ocean will result in even greater loss of smolt characteristics and in a larger portion of the population being affected. The proportion of the population affected by loss of smolt characteristics will depend on the timing of migration of the population and the temperature experienced throughout the migratory period. Dams and their impoundments will increase the problem of lost smolt characteristics by delaying migration and increasing water temperatures. In regulated river systems, loss of smolt characteristics can be reduced by ensuring that smolts have the opportunity to maximize migratory rate, by ensuring safe, efficient, and timely passage of fish over or through dams. To increase the effectiveness of resource management, information on loss of smolt characteristics and reduced smolt survival could be combined with adaptive management strategies. Such management actions may be particularly important in years with unusually rapid rising or high spring temperatures or in mitigating the effects of global climate change.

The strong correspondence between the loss of salinity tolerance and decreased gill Na^+,K^+-ATPase activity suggests a mechanism by which temperature may exert its influence on salinity tolerance. Gill Na^+,K^+-ATPase activity is located primarily in chloride cells, which are responsible for ion secretion in seawater (Karnaky et al. 1976; Foskett and Scheffey 1982). Chloride cell density increases along with gill Na^+,K^+-ATPase activity during smolting (Langdon and Thorpe 1985; Lubin et al. 1991), and the decrease in gill Na^+,K^+-ATPase after the peak of smolting indicates that the number of chloride cells may also decrease. Higher temperatures may result in higher "turnover" or cell death of chloride cells, decreased renewal of chloride cells, or both. The effect of temperature may also be less direct, perhaps differentially affecting endocrine systems promoting or inhibiting the maintenance of chloride cells and salinity tolerance.

Some of the variability that we observed in salinity tolerance may have been due to stress caused by transport of stream-reared fish and their exposure to a laboratory environment. Such stress may be inherent to studies in which fish from the wild are tested under laboratory conditions. We have found that laboratory-reared fish subjected to handling and confinement stress do not show decreased salinity tolerance (S.D. McCormick, unpublished results). This stress, however, may not be similar to that experienced by fish from the wild that have never been subjected to handling or confinement. Gill Na^+,K^+-ATPase activity in fish sampled immediately upon capture will not be affected by stress and may be a more accurate representation of changes in smolt development.

There is little information on the degree to which migratory behavior is lost and how it is affected by high temperature or how tight is the coupling between smolt physiology and behavior. Greenstreet (1992) found that movement rates of individually tagged Atlantic salmon smolts migrating through a fish ladder increased with increasing temperature up to 16°C but decreased slightly at 16–18°C. Studies of Pacific salmon show a close correspondence between physiological smolt development and migratory behavior (Zaugg et al. 1985; Zaugg 1989; Ewing et al. 1994), although these studies did not explicitly examine loss of smolt characteristics. If there is a strong link between migratory behavior and gill Na^+,K^+-ATPase activity in Atlantic salmon, migratory behavior will decrease in late spring and will be lost more rapidly at high temperatures. If the link is not strong, smolts with reduced gill Na^+,K^+-ATPase activity may continue to migrate to the estuary. It is likely that they will experience substantial osmotic disequilibrium or spend substantially more time acclimating to seawater than normal smolts, making them more likely to suffer higher predation rates (Handeland et al. 1996).

Gill Na^+,K^+-ATPase activity was low in smolts early in migration in northern rivers (Catamaran Brook and the Conne River), intermediate in smolts from the Penobscot River, and highest in smolts from the Connecticut River, roughly corresponding to the early spring temperature regimes for the four rivers. There are also temperature-related differences within rivers. In the Conne River the greatest gill Na^+,K^+-ATPase activity at the first sampling period occurred in 1995, the warmest of the three years examined. In the Connecticut River the only year in which significant increases occurred early in migration was 1997, which had the coolest spring temperatures. Smolts sampled in tributaries of the Connecticut River have lower gill Na^+,K^+-ATPase activity than smolts at Cabot Station (S.D. McCormick, unpublished observation), indicating that the peak levels are reached in the mainstem. Previous research has shown that

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the timing of increased salinity tolerance and other aspects of smolting are most strongly influenced by changes in daylength and that temperature plays a less important role in the onset of physiological smolt characteristics (Johnston and Saunders 1981; Clarke et al. 1985; Duston and Saunders 1990). The present findings suggest that temperature may play a role in the initial (early spring) phase of smolt physiology.

In spite of the fairly large differences in early spring temperatures among the rivers, there was general similarity in the migratory period of Atlantic salmon. Almost all of the migration in each year began after May 1 and was completed by the first week of June. In most years the date of 50% migration was quite similar among the rivers. The importance of photoperiod in initiating migration may account for the similarity in run timing among these rivers. There was variation in run timing within rivers that was correlated with spring temperatures. In the Connecticut River, the earliest and latest days of 50% migration were in 1993 (May 12) and 1997 (May 23), the warmest and coolest springs, respectively (Fig. 1). Similarly, the date of 50% completion of the smolt migration in the Conne River was May 3, 1996, when there was an early increase in temperatures, whereas a cool spring the subsequent year was associated with 50% migration on May 26 (Fig. 3).

We have demonstrated that migrating Atlantic salmon smolts have initially high salinity tolerance and gill Na^+,K^+-ATPase activity and that reductions in these physiological smolt characteristics occur at the end of the normal migratory period in southern populations that experience high temperatures in late spring. Loss of physiological smolt characteristics did not occur in more northern rivers. Because the loss of smolt characteristics is related to degree-days and not just absolute temperature, time of migration will also be a factor in determining how many smolts will be affected. Thus, factors other than just temperature, such as hydrographic and climatic conditions and barriers to migration such as dams, will also be important. In warm southern rivers, significant delays in migration will have negative impacts on the capacity of smolts to survive in seawater and return as adults.

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References

Adams, B.L., Zaugg, W.S., and Mclain, L.R. 1973. Temperature effect on parr–smolt tranformation in steelhead trout (*Salmo gairdneri*) as measured by gill sodium–potassium stimulated ATPase. Comp. Biochem. Physiol. A, Comp. Physiol. **44**: 1333–1339.

Clarke, W.C., Lundqvist, H., and Eriksson, L.O. 1985. Accelerated photoperiod advances seasonal cycle of seawater adaptation in juvenile Baltic salmon, *Salmo salar* L. J. Fish Biol. **26**: 29–35.

Cunjak, R.A., and Therrien, J. 1997. Inter-stage survival of wild juvenile Atlantic salmon, *Salmo salar* L. Fish. Manage. Ecol. **5**: 209–223.

Dempson, J.B., and Stansbury, D.E. 1991. Using partial counting fences and a two-sample stratified design for mark–recapture estimation of an Atlantic salmon smolt population. N. Am. J. Fish. Manage. **11**: 27–37.

Dempson, J.B., Furey, G., and Bloom, M. 1998. Status of Atlantic salmon in Conne River, SFA 11, Newfoundland, 1997. Can. Stock Asses. Secr. Res. Doc. 98/28.

Duston, J., and Saunders, R.L. 1990. The entrainment role of photoperiod on hypoosmoregulatory and growth-related aspects of smolting in Atlantic salmon (*Salmo salar*). Can. J. Zool. **68**: 707–715.

Duston, J., Saunders, R.L., and Knox, D.E. 1991. Effects of increases in freshwater temperature on loss of smolt characteristics in Atlantic salmon (*Salmo salar*). Can. J. Fish. Aquat. Sci. **48**: 164–169.

Ewing, R.D., Barratt, D., and Garlock, D. 1994. Physiological changes related to migration tendency in rainbow trout (*Oncorhynchus kisutch*). Aquaculture, **121**: 277–287.

Foskett, J.K., and Scheffey, C. 1982. The chloride cell: definitive identification as the salt-secretory cell in teleosts. Science (Washington, D.C.), **215**: 164–166.

Greenstreet, S.P.R. 1992. Migration of hatchery reared juvenile Atlantic salmon, *Salmo salar* L., smolts down a release ladder. 1. Environmental effects on migratory activity. J. Fish Biol. **40**: 655–666.

Handeland, S.O., Jarvi, T., Ferno, A., and Stefansson, S.O. 1996. Osmotic stress, antipredator behaviour, and mortality of Atlantic salmon (*Salmo salar*) smolts. Can. J. Fish. Aquat. Sci. **53**: 2673–2680.

Hansen, L.P. 1987. Growth, migration and survival of lake reared juvenile anadromous Atlantic salmon *Salmo salar* L. Fauna Norv. Ser. A, **8**: 29–34.

Hoar, W.S. 1988. The physiology of smolting salmonids. *In* Fish physiology. Vol. XIB. *Edited by* W.S. Hoar and D. Randall. Academic Press, New York. pp. 275–343.

Johnston, C.E., and Saunders, R.L. 1981. Parr–smolt transformation of yearling Atlantic salmon (*Salmo salar*) at several rearing temperatures. Can. J. Fish. Aquat. Sci. **38**: 1189–1198.

Karnaky, K.J., Kinter, L.B., Kinter, W.B., and Stirling, C.E. 1976. Teleost chloride cell. Autoradiographic localization of gill Na,K-ATPase in killifish *Fundulus heteroclitus* adapted to low and high salinity environments. J. Cell Biol. **70**: 157–177.

Langdon, J.S., and Thorpe, J.E. 1985. The ontogeny of smoltification: developmental patterns of gill Na^+/K^+-ATPase, SDH, and chloride cells in juvenile Atlantic salmon, *Salmo salar* L. Aquaculture, **45**: 83–96.

Lubin, R.T., Rourke, A.W., and Saunders, R.L. 1991. Influence of photoperiod on the number and ultrastructure of gill chloride cells of the Atlantic salmon (*Salmo salar*) before and during smoltification. Can. J. Fish. Aquat. Sci. **48**: 1302–1307.

McCormick, S.D. 1993. Methods for nonlethal gill biopsy and

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measurement of Na^+,K^+-ATPase activity. Can. J. Fish. Aquat. Sci. **50**: 656–658.

McCormick, S.D., and Björnsson, B.T. 1994. Physiological and hormonal differences among Atlantic salmon parr and smolts reared in the wild, and hatchery smolts. Aquaculture, **121**: 235–244.

McCormick, S.D., Shrimpton, J.M., and Zydlewski, J.D. 1997. Temperature effects on osmoregulatory physiology of juvenile anadromous fish. *In* Global warming: implications for freshwater and marine fish. *Edited by* C.M. Wood and D.G. McDonald. Cambridge University Press, New York. pp. 279–301.

McCormick, S.D., Saunders, R.L., Hansen, L.P., and Quinn, T.P. 1998. Movement, migration, and smolting in Atlantic salmon (*Salmo salar*). Can. J. Fish. Aquat. Sci. **55**(Suppl.1): 77–92.

McMenemy, J.R. 1995. Survival of Atlantic salmon fry stocked at low density in the West River, Vermont. N. Am. J. Fish. Manage. **15**: 366–374.

Moffitt, C.M., Kynard, B., and Rideout, S.G. 1982. Fish passage facilities and anadromous fish restoration in the Connecticut River basin. Fisheries (Bethesda), **7**: 2–11.

Orciari, R.D., and Leonard, G.H. 1996. Length characteristics of smolts and timing of downstream migration among three strains of Atlantic salmon in a southern New England. N. Am. J. Fish. Manage. **16**: 851–860.

Rideout, S.G., and Stolte, L.W. 1988. Restoration of Atlantic salmon to the Connecticut and Merrimack rivers. *In* Present and future atlantic salmon management. *Edited by* R.H. Stroud. Atlantic Salmon Federation, Ipswich, Mass. pp. 67–81.

Staurnes, M., Lysfjord, G., Hansen, L.P., and Heggberget, T.G. 1993. Recapture rates of hatchery-reared Atlantic salmon (*Salmo salar*) related to smolt development and time of release. Aquaculture, **118**: 327–337.

Virtanen, E., Soderholmtana, L., Soivio, A., Forsman, L., and Muona, M. 1991. Effect of physiological condition and smoltification status at smolt release on subsequent catches of adult salmon. Aquaculture, **97**: 231–257.

Whitesel, T.A. 1993. Comparison of juvenile Atlantic salmon (*Salmo salar*) reared in a hatchery and introduced into a stream: a two-size-threshold model of smoltification. Can. Spec. Publ. Fish. Aquat. Sci. No. 118. pp. 239–247.

Zaugg, W.S. 1981. Advanced photoperiod and water temperature effects on gill Na^+-K^+ adenosine triphosphatase activity and migration of juvenile steelhead (*Salmo gairdneri*). Can. J. Fish. Aquat. Sci. **38**: 758–764.

Zaugg, W.S. 1989. Migratory behaviour of underyearling *Oncorhynchus tshawytscha* and survival to adulthood as related to prerelease gill (Na^+-K^+)-ATPase development. Aquaculture, **82**: 339–353.

Zaugg, W.S., and McLain, L.R. 1976. Influence of water temperature on gill sodium, potassium-stimulated ATPase activity in juvenile coho salmon (*Oncorhynchus kisutch*). Comp. Biochem. Physiol. A, Comp. Physiol. **54**: 419–421.

Zaugg, W.S., Prentice, E.F., and Waknitz, F.W. 1985. Importance of river migration to the development of seawater tolerance in Columbia river anadromous salmonids. Aquaculture, **51**: 33–47.

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Genetic structure and differentiation among populations of two cyprinids, *Leuciscus cephalus* and *Rutilus rutilus*, in a large European river

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Abstract: The aim of this investigation was to compare the genetic responses of two common cyprinids, chub (*Leuciscus cephalus*) and roach (*Rutilus rutilus*), to various habitat and pollution regimes within the same river basin, the Rhone, using allozyme data. Over the three main portions of the River Rhone, lower genetic polymorphism and lower heterozygosity were detected for the chub in the Saone, which is characterized by lower environmental diversity than in the Lower and Upper Rhone. On a local scale, no detectable decrease in polymorphism or heterozygosity was observed in populations of both species living in heavily polluted sites of the Rhone's Chemical Corridor. Significant differentiation was detected between populations of both chub and roach localized upstream and downstream from the dams of Donzère Mondragon, which were erected 45 years ago. The *F* statistics and neighbor-joining analysis of genetic distances suggested that the Upper Rhone populations were unique in comparison with the other populations for both species; this situation could be the result of historical factors and (or) a selective pressure in a variable environment. Isolation by distance was observed for chub in the less perturbed river system over a range of 100 km. By contrast, there was no relationship between geographic distance and genetic distance for roach, whatever the subsystem tested.

Résumé : L'objectif de ce travail est de comparer la réponse génétique de deux cyprinidés communs, le Chevaine (*Leuciscus cephalus*) et le Gardon (*Rutilus rutilus*), à des situations d'habitat et de poliution variables au sein du même bassin fluvial, le Rhône, en analysant le polymorphisme enzymatique. Sur les trois sous-systèmes du Rhône, le plus faible polymorphisme génétique et la plus faible hétérozygotie ont été détectés pour le Chevaine en Saône, zone présentant une diversité environnementale nettement plus réduite par rapport au Haut et Bas Rhône. A une échelle locale, aucune baisse du polymorphisme ou de l'hétérozygotie n'a été détectée pour les populations des deux espèces dans les sites fortement pollués du couloir chimique Rhodanien. Des divergences génétiques ont été détectées entre les populations de Chevaine et Gardon situées en amont et en aval des barrages de Donzère Mondragon, vieux de 45 ans. Par les statistiques *F* et l'analyse des distances génétiques par « neighbor-joining », l'originalité des populations du Haut Rhône a été mise en évidence par rapport aux autres populations, pour les deux espèces; cette situation pourrait être le résultat de facteurs historiques et (ou) d' une pression sélective s'exerçant dans un environnement variable. Une tendance à l'isolement par la distance a été observée pour le Chevaine, dans le sous-système le moins perturbé, sur 100 km; aucune relation entre distance géographique et distance génétique n'a été mise en évidence pour le Gardon, quel que soit le sous-système testé.

Introduction

At present, most of the world's large river systems show extensive damming, regulation, and diversion, which lead to the disappearance of habitats and to the obstruction to organism dipersal. Theoretically, the fragmentation of habitats could induce an erosion of the genetic variability within populations (via bottleneck effect, genetic drift, inbreeding) and an increase in the divergence among populations by reducing gene flow. Empirical studies with natural populations are necessary to investigate such complex processes (Young et al. 1996).

The subdivision of fish populations in fluvial networks can be observed on different geographic scales (McClenaghan et al. 1985). Previous analysis of genetic structure performed on different fish species in the same river system stressed two major types of factors explaining the fragmentation of populations (Tibbets and Dowling 1996; Heithaus and Laushman 1997): intrinsic factors that could affect gene flow (vagility, reproduction behavior, niche width) and extrinsic factors in both the long term (geological history) and the short term (loss of habitats induced by man, water quality).

In European rivers, some species of cyprinid fish are distributed widely from undisturbed to heavily perturbed sites and offer numerous possibilities to explore the structure of

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genetic diversity in contrasting situations. The cyprinids display large relative fecundity, a great number of reproductive cycles in comparison with salmonids (Pont et al. 1995), and probably a high total lifetime fecundity per unit biomass. This reproductive strategy associated with a relatively large niche width for the majority of them could explain the high genetic variability within populations (Mitton and Lewis 1989). The recent literature on the genetics of common cyprinids in North European basins, roach (*Rutilus rutilus*), chub (*Leuciscus cephalus*), and nase (*Chondrostoma nasus*) (Bouvet et al. 1995; Guinand et al. 1996; Baranyi et al. 1997; Gollmann et al. 1997; Hänfling and Brandl 1998; Wolter 1998), has confirmed that the genetic variability within populations was generally higher than the genetic variability between populations. In the south of Europe, and particularly in the Iberian Peninsula, some small populations of endemic cyprinids had a restricted habitat distribution and consequently showed reduced heterozygosity (Coelho et al. 1995).

The impact of anthropogenic pressure effects of contaminants and stream regulation on the genetic variation of freshwater fish was investigated by analysing the frequencies of allozymes in natural populations. Individuals with certain genotypes may be more sensitive to the toxic effects of polluted waters than those with other genotypes, and selection may result in a narrowing of the genetic diversity (Gillespie and Guttman 1993; Heithaus and Laushman 1997). Stream regulation may create obstacles to fish migration and may affect stream flow, water quality, and thermal regimes. Fish subjected to unpredictable thermal regimes associated with dams used to produce hydroelectric power may exhibit an increase in genetic variation (Zimmerman and Richmond 1981). Lower levels of genetic variation in fish populations below a lock and dam have been reported (Smith at al. 1983), and the presence of long canals can constitute migration barriers for fish, affecting gene flow (Wolter 1998).

The aim of this investigation was to compare the genetic responses of two common cyprinid species (chub and roach) to various habitat and pollution regimes within the same river basin, the Rhone, using allozyme data. The chub data were previously exploited by Guinand et al. (1996) using factorial analysis, while the roach data are presented here. These two species display a relatively high longevity and start reproducing at the age of 2 or 3, once a year, with a maximum of 11 and 17 reproductive cycles for roach and chub, respectively (Pont et al. 1995). They show flexibility in spawning habitats, feeding, and refuge (Mann 1996); chub and roach are lithophil and phytolithophil spawners, respectively, and are considered omnivorous. These species are widely distributed over the Rhone system; chub are considered an ubiquitous species with a slight rheophilic preference, and roach appear to be more lentic (Carrel and Rivier 1996). Both species differ in their microhabitats rather than in their overall distribution; chub are present as well in the swiftly flowing channels as in some side arms, reservoirs, and lakes, whereas roach thrive in quiet waters (slow rivers, side arms, reservoirs, lakes).

Based on mean annual discharge, the Rhone is noted as the 42nd largest river in the world and some sectors are heavily perturbed by anthropogenic pressure from 19 hydroelectric plants, five nuclear plants, and industrial pollution (Bravard et al. 1992). This system offers the possibility to investigate the genetic structure of fish populations in relation to different spatial scales and various anthropogenic pressures.

Materials and methods

The Rhone river can be divided into three main subsystems (Pattée 1988). The Saone (mean annual discharge 400 $m^3 \cdot s^{-1}$) and its left bank tributaries (Seille, Reyssouse) flow slowly (current velocity: 0–0.5 $m \cdot s^{-1}$) through the Bresse plain (Fig. 1). Its right bank tributary, the Grosne, is more lotic because of the hilly landscape of its upstream section.

The French Upper Rhone (mean annual discharge 360 $m^3 \cdot s^{-1}$), between Lake Geneva and its confluence with the Saone in the city of Lyon, cuts through the Jura Mountain Range. Current flow is generally swift (0–2 $m \cdot s^{-1}$) (Pattée 1988; Bravard et al. 1992). The Génissiat Dam was completed in 1951, and downstream from it, the Rhone was modified with five recent dams (starting with Chautagne Dam in 1981), which are too recent to have influenced fish genotypes (not presented in Fig. 1).

The last subsystem, the Lower Rhone, flows below the city of Lyon. It includes stations Cb1, Rb1, and Rb2 with an average discharge of 800 $m^3 \cdot s^{-1}$ (Fig. 1) and is characterized by domestic inputs from the city and industrial inputs from a high concentration of chemical factories (Chemical Corridor). The whole Lower Rhone includes a succession of 12 dams built between 1948 and 1981 with several nuclear power plants (Pattée 1988; Fruget 1992). The original hydropower development scheme included construction of a diversion canal parallel to the old channel, which was closed by a diversion dam. The longitudinal profile of the Lower Rhone is now a succession of reservoirs and diversion canals with low slopes displaying a reduced flow velocity during medium flow. The old channel (bypassed section) only receives a very small compensation flow (10 or 20 $m^3 \cdot s^{-1}$), but flow increases greatly during floods when the capacity of the diversion canal is exceeded.

Stations Cb2 (chub) and Rb3 (roach) are separated from downstream stations by two dams (Fig. 1): the diversion dam above the bypassed section on the western side and the hydroelectric dam and lock on the newly built canal (headrace and tailrace) on the eastern side. This first dam system (Donzère Mondragon) was erected on the Rhone in 1952 and includes a nuclear plant erected in 1981. In this sector, total discharge is about 1700 $m^3 \cdot s^{-1}$ and the headrace is perturbed by a discharge of heated water from the nuclear plant (170 $m^3 \cdot s^{-1}$ full power), which may increase the temperature locally from 3 to 9°C (Carrel and Rivier 1996). Stations Cb3, Cb6, Rb4, and Rb7 are localized on the bypassed section and stations Cb4 and Rb5 are localized on the tailrace. The sampling program included one station (Cb5, Rb6) on a tributary of the bypassed section, the River Ardèche.

Major pollutants in the different compartments of the Rhone system (organic matter, total nitrogen, total phosphorus, heavy metals, polychlorinated biphenyls, polycyclic aromatic hydrocarbons) were analysed (Agence de Bassin Rhône–Méditerranée–Corse 1998). From these results, stream water quality can be generalized in the following order: Upper Rhone > Saone > Lower Rhone (without stations Cb1, Rb1, Rb2) > Chemical Corridor (Cb1, Rb1, Rb2). The improvement of the chemical quality of water in the sector of Donzère Mondragon is probably linked to dilution and self-purification effects downstream from the Chemical Corridor.

Samples of chub and roach were collected by electrofishing from 1991 to 1994 (Fig. 1). Twenty-seven and 23 populations were sampled for chub and roach, respectively, in the whole Rhone basin. Fishes were brought on ice to the laboratory and frozen at –80°C. Muscle and liver extracts were prepared, and zymograms were obtained by migration on starch gels according to Bouvet et al. (1995). Twenty-eight and 26 loci were analysed for chub (c) and

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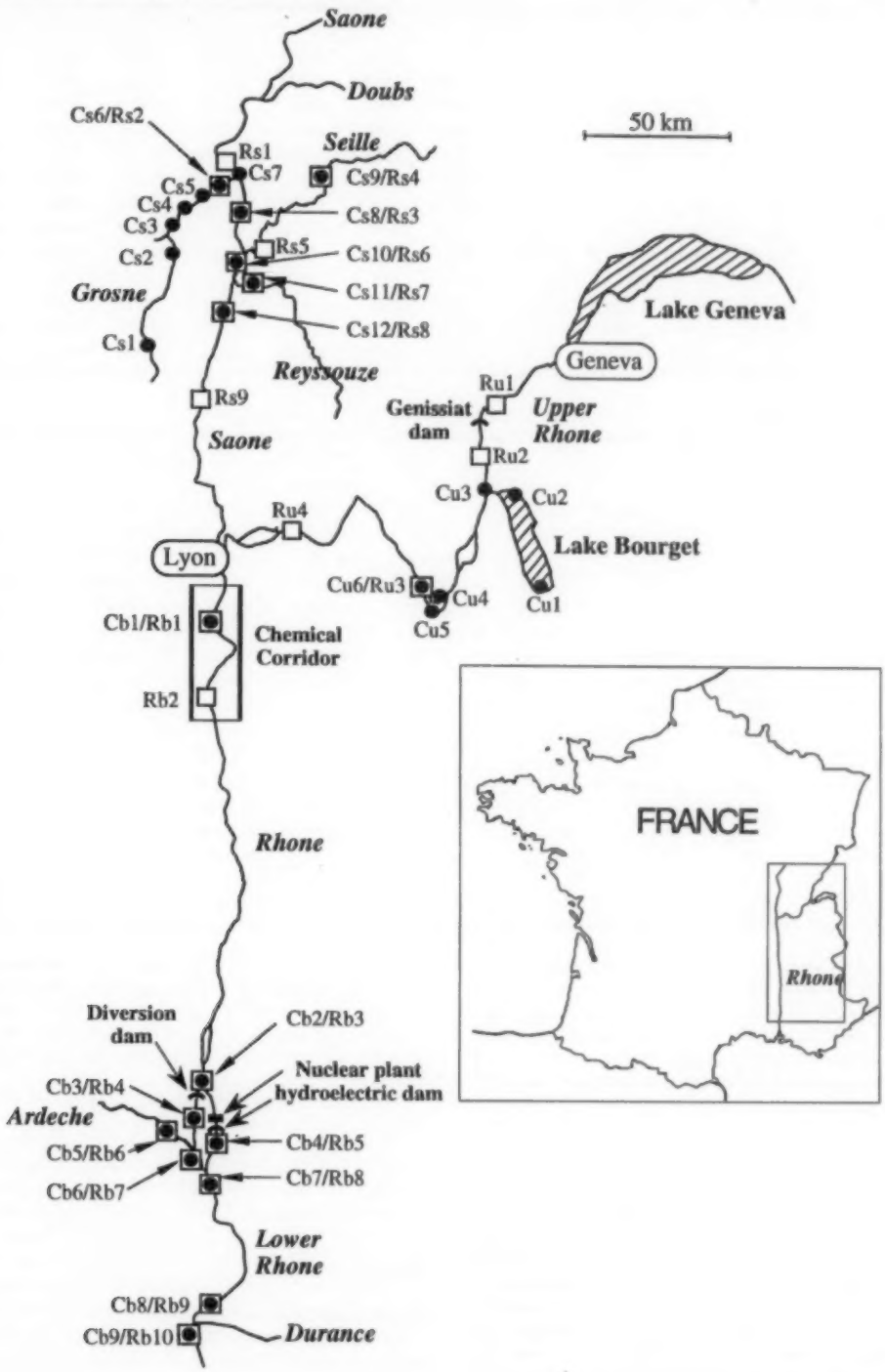
Fig. 1. Rhone River and the sampling sites for chub and roach.



roach (r) populations, respectively. These were, following the nomenclature of Shaklee et al. (1990): *AAT-1* (c,r), *AAT-2* (c,r), *AK* (c,r), *CK-1* (c), *CK* (r), *EST-1* (c), *EST-2* (c), *FH* (c), *GAPDH* (r), *GDA* (r), *G3PDH* (c,r), *G6PDH* (r), *GPI-1* (c,r), *GPI-2* (c,r), *IDDH* (c,r), *IDHP-1* (c,r), *IDHP-2* (c,r), *IDHP-3* (c), *LDH-1* (c,r), *LDH-2* (c,r), *LDH-3* (c,r), *MDH-1* (c,r), *MDH-2* (c,r), *MDH-3* (c,r), *MEP-1* (c), *MEP-2* (c), *MEP* (r), *MPI* (c), *MPI-1* (r), *MPI-2* (r), *PGDH* (c,r), *PGM-1* (c,r), *PGM-2* (c,r), *SOD* (c,r), and *XDH* (c).

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Table 1. Polymorphic loci, alleles, and allele frequencies in the populations of chub examined.

		Cs1	Cs2	Cs3	Cs4	Cs5	Cs6	Cs7	Cs8	Cs9	Cs10	Cs11	Cs12
PGM-2	*90*	0.017	0.083	0.107	0.064	0.103	0.192	0.196	0.250	0.286	0.175	0.154	0.125
	98	0.100	0.217	0.179	0.173	0.190	0.135	0.196	0.115	0.000	0.050	0.039	0.104
	100	0.883	0.700	0.714	0.764	0.707	0.673	0.607	0.635	0.714	0.775	0.808	0.771
IDHP-1	*100*	1.000	1.000	0.982	1.000	1.000	0.981	0.982	1.000	0.976	0.975	0.962	0.979
	145	0.000	0.000	0.018	0.000	0.000	0.019	0.018	0.000	0.024	0.025	0.039	0.021
EST-2	*98*	0.300	0.300	0.250	0.336	0.362	0.250	0.232	0.308	0.310	0.250	0.308	0.354
	100	0.700	0.700	0.750	0.664	0.638	0.750	0.768	0.692	0.691	0.750	0.692	0.646
G3PDH	*85*	0.200	0.167	0.179	0.146	0.103	0.154	0.089	0.039	0.119	0.050	0.154	0.104
	100	0.800	0.833	0.821	0.855	0.897	0.846	0.911	0.962	0.881	0.950	0.846	0.896
	n	30	30	28	55	29	26	28	26	21	20	13	24
	$P > 95\%$	0.107	0.107	0.107	0.107	0.107	0.107	0.107	0.071	0.107	0.107	0.107	0.107
	H_o	0.037	0.036	0.045	0.037	0.038	0.037	0.048	0.032	0.046	0.036	0.038	0.037
	H_e	0.034	0.041	0.041	0.039	0.039	0.042	0.040	0.036	0.039	0.032	0.039	0.038

Table 2. Polymorphic loci, alleles and allele frequencies in the populations of Roach examined.

		Rs1	Rs2	Rs3	Rs4	Rs5	Rs6	Rs7	Rs8	Rs9
AAT-2	*90*	0.472	0.411	0.467	0.467	0.476	0.517	0.339	0.417	0.407
	100	0.528	0.589	0.533	0.533	0.524	0.483	0.661	0.583	0.593
G6PDH	*70*	0.222	0.286	0.283	0.350	0.405	0.350	0.304	0.217	0.315
	90	0.222	0.196	0.167	0.117	0.095	0.133	0.179	0.233	0.167
	95	0.000	0.018	0.000	0.033	0.000	0.000	0.000	0.000	0.000
	100	0.556	0.500	0.550	0.500	0.500	0.517	0.518	0.550	0.519
GPI-1	*100*	0.944	0.964	0.983	0.967	1.000	0.983	0.982	0.983	0.954
	105	0.056	0.036	0.017	0.033	0.000	0.017	0.018	0.017	0.046
GPI-2	*100*	0.972	1.000	1.000	1.000	0.976	0.983	1.000	1.000	1.000
	115	0.028	0.000	0.000	0.000	0.024	0.017	0.000	0.000	0.000
PGM-2	*95*	0.000	0.018	0.017	0.000	0.024	0.017	0.000	0.000	0.037
	100	0.917	0.875	0.883	0.817	0.857	0.933	0.893	0.767	0.759
	103	0.083	0.107	0.100	0.183	0.119	0.050	0.107	0.233	0.204
MPI-2	*88*	0.056	0.036	0.017	0.083	0.000	0.050	0.054	0.017	0.037
	100	0.944	0.964	0.983	0.917	1.000	0.950	0.946	0.983	0.963
	n	18	28	30	30	21	30	28	30	54
	$P > 95\%$	0.192	0.115	0.115	0.154	0.115	0.154	0.154	0.115	0.115
	H_o	0.064	0.054	0.053	0.068	0.057	0.054	0.047	0.062	0.056

Observed heterozygosity (H_o), average heterozygosity based on Hardy–Weinberg expectations (H_e), and percentage of polymorphic loci ($P > 95\%$) were computed for the different populations. Analyses of variance (ANOVA) and Student–Newman–Keuls (SNK) analyses using arcsine square root transformed values of H_o or P were carried out to compare the different Rhone subsystems. Allele and genotype frequencies were analysed by χ^2 contingency tests to assess heterogeneity among populations upstream and downstream from the dams. Fixation indices were computed by the procedure of Weir and Cockerham (1984). Tests of significance of F_{is} and F_{st} were carried out by permutation. Permutated data sets (5000) were generated from an original data set; for F_{is}, alleles were permuted within samples, and for F_{st}, individual multilocus genotypes were permuted among samples. The F_{is} was calculated for each permutated file, generating a distribution of F_{is} values corresponding to the null hypothesis that $F_{is} = 0$. The chance probability of obtaining the original F_{is} value was calculated as the proportion of the distribution having F_{is} values larger than the original value. Genetic distances between pairs of populations were computed according to Reynolds et al. (1983). The correlation between genetic distances and geographic distances was examined using the Mantel test to detect a possible isolation by distance between the populations. This correlation was successively computed for chub and roach along three river continuums: Grosne–Saone, Lower Rhone following the bypassed section, and Lower Rhone following the diversion canal. The Mantel test was not carried out on the Upper Rhone because the number of stations was too low. All treatments were performed using the computer package GENETIX 3.0 (Belkhir et al. 1996).

The genetic relationship among samples was described by performing a neighbor-joining analysis of genetic distances (Nei 1978) using the appropriate routines within PHYLIP (Felsenstein 1993). NEIGHBOR constructs a tree by successive clustering of lineages. The tree does not assume an evolutionary clock, so that it is in effect an unrooted tree.

Results

Population genetic variation

Allelic frequencies were computed for chub ($n = 822$) and roach ($n = 687$) (Tables 1 and 2). Four loci (*PGM-2*, *IDHP-1*, *EST-2*, *G3PDH*) out of 28 and six loci (*AAT-2*, *G6PDH*, *GPI-1*, *GPI-2*, *PGM-2*, *MPI-2*) out of 26 were polymorphic for chub and roach, respectively (Tables 1 and 2). Chub polymorphism ($P > 95\%$) varied from 7 to 14%, and roach

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Cu1	Cu2	Cu3	Cu4	Cu5	Cu6	Cb1	Cb2	Cb3	Cb4	Cb5	Cb6	Cb7	Cb8	Cb9
0.000	0.031	0.000	0.040	0.033	0.033	0.129	0.155	0.138	0.177	0.125	0.119	0.135	0.088	0.059
0.500	0.592	0.691	0.620	0.583	0.683	0.343	0.138	0.103	0.059	0.089	0.143	0.096	0.103	0.118
0.500	0.377	0.310	0.340	0.383	0.283	0.529	0.707	0.759	0.765	0.786	0.738	0.769	0.809	0.824
0.583	0.715	0.691	0.740	0.750	0.800	0.871	0.948	0.966	0.941	0.893	0.881	0.923	0.912	0.941
0.417	0.285	0.310	0.260	0.250	0.200	0.129	0.052	0.035	0.059	0.107	0.119	0.077	0.088	0.059
0.317	0.423	0.369	0.460	0.300	0.317	0.300	0.276	0.241	0.250	0.339	0.405	0.308	0.338	0.353
0.683	0.577	0.631	0.540	0.700	0.683	0.700	0.724	0.759	0.750	0.661	0.595	0.692	0.662	0.647
0.067	0.108	0.119	0.140	0.083	0.167	0.086	0.052	0.121	0.162	0.054	0.048	0.115	0.103	0.074
0.933	0.892	0.881	0.860	0.917	0.833	0.914	0.948	0.879	0.838	0.946	0.952	0.885	0.897	0.927
30	65	42	25	30	30	35	29	29	34	28	21	26	34	34
0.143	0.143	0.143	0.143	0.143	0.143	0.143	0.143	0.107	0.143	0.143	0.107	0.143	0.143	0.143
0.060	0.051	0.050	0.066	0.058	0.046	0.045	0.044	0.036	0.036	0.037	0.046	0.041	0.047	0.038
0.055	0.057	0.055	0.058	0.052	0.053	0.050	0.038	0.037	0.041	0.039	0.043	0.041	0.040	0.036

Ru1	Ru2	Ru3	Ru4	Rb1	Rb2	Rb3	Rb4	Rb5	Rb6	Rb7	Rb8	Rb9	Rb10
0.633	0.683	0.467	0.417	0.293	0.500	0.300	0.433	0.350	0.433	0.450	0.467	0.433	0.500
0.367	0.317	0.533	0.583	0.707	0.500	0.700	0.567	0.650	0.567	0.550	0.533	0.567	0.500
0.583	0.683	0.517	0.383	0.293	0.350	0.300	0.300	0.283	0.250	0.283	0.383	0.233	0.241
0.067	0.117	0.167	0.083	0.172	0.150	0.167	0.100	0.200	0.117	0.117	0.083	0.117	0.172
0.000	0.000	0.050	0.100	0.000	0.017	0.000	0.000	0.017	0.017	0.033	0.033	0.000	0.000
0.350	0.200	0.267	0.433	0.535	0.483	0.533	0.600	0.500	0.617	0.567	0.500	0.650	0.586
1.000	1.000	1.000	0.967	0.966	1.000	0.983	0.983	0.983	1.000	0.983	0.933	0.933	0.983
0.000	0.000	0.000	0.033	0.035	0.000	0.017	0.017	0.017	0.000	0.017	0.067	0.067	0.017
1.000	0.950	0.983	0.983	1.000	0.983	1.000	0.983	1.000	1.000	1.000	1.000	1.000	1.000
0.000	0.050	0.017	0.017	0.000	0.017	0.000	0.017	0.000	0.000	0.000	0.000	0.000	0.000
0.033	0.000	0.117	0.000	0.000	0.033	0.033	0.000	0.000	0.000	0.000	0.000	0.033	0.000
0.883	0.933	0.833	0.900	0.879	0.850	0.867	0.767	0.900	0.883	0.900	0.883	0.817	0.828
0.083	0.067	0.050	0.100	0.121	0.117	0.100	0.233	0.100	0.117	0.100	0.117	0.150	0.172
0.250	0.400	0.233	0.117	0.052	0.083	0.083	0.000	0.033	0.050	0.033	0.050	0.050	0.069
0.750	0.600	0.767	0.883	0.948	0.917	0.917	1.000	0.967	0.950	0.967	0.950	0.950	0.931
30	30	30	30	29	30	30	30	30	30	30	30	30	29
0.154	0.192	0.154	0.154	0.154	0.154	0.154	0.115	0.115	0.154	0.115	0.192	0.192	0.154
0.060	0.062	0.063	0.058	0.056	0.065	0.054	0.053	0.050	0.051	0.049	0.058	0.068	0.064

polymorphism varied from 12 to 19% (Tables 1 and 2). The distribution of polymorphism over the three main geographic districts was heterogenous for chub (mean Saone P < mean Lower Rhone P ≈ mean Upper Rhone P; ANOVA and SNK with $p < 0.01$: 10.4 < 13.5 ≈ 14.3) and homogenous for roach (mean Saone P ≈ mean Lower Rhone P ≈ mean Upper Rhone P; ANOVA with $p > 0.05$: 13.6 ≈ 14.9 ≈ 16.4).

Observed heterozygosity varied from 0.032 to 0.066 (mean H_o = 0.043) for chub and from 0.047 to 0.068 (mean H_o = 0.057) for roach (Tables 1 and 2). For chub, the distribution of H_o over the whole basin displayed a convergent pattern with the one observed for polymorphism (mean Saone H_o ≈ mean Lower Rhone H_o < mean Upper Rhone H_o; ANOVA and SNK with $p < 0.01$: 0.039 ≈ 0.041 < 0.055). The distribution of H_o was approximately uniform for roach over the three areas.

Over the Lower Rhone region, the lowest P values were observed for both species downstream from the two dams (Cb3 = Cb6 = 10.7%; Rb4 = Rb5 = Rb7 = 11.5%) (Fig. 1). The same trend was detected for chub heterozygosity, the lowest H_o values being observed downstream from the two dams (Cb3 = Cb4 = Cb5 = 0.036). The three samples for chub (Cb2, Cb3, Cb4) and for roach (Rb3, Rb4, Rb5) were tested for differences in allele frequencies and in genotype frequencies in order to investigate the potential impact of the two dams on the fish populations. For roach, the allele frequencies at the locus *PGM-2* were significantly different between Rb3 and Rb4 (the counts of the rare *95* allele were pooled with those of *100*; $\chi^2 = 3.840$, 1 df, $p < 0.05$) and not significantly different between Rb3 and Rb5. No significant difference was detected in allele frequencies at any locus for chub, and the heterogeneity was observed between genotype frequencies. The frequencies of chub with the genotypes *EST-2 100/100* and *EST-2 100/98* were significantly different between Cb2 and Cb4 ($\chi^2 = 3.827$, 1 df, $p < 0.05$), heterozygotes being less numerous downstream from the hydroelectric dam and lock; no difference was detected for this locus between Cb2 and Cb3.

F statistics and neighbor-joining analysis

Over the whole Rhone basin, differentiation between the

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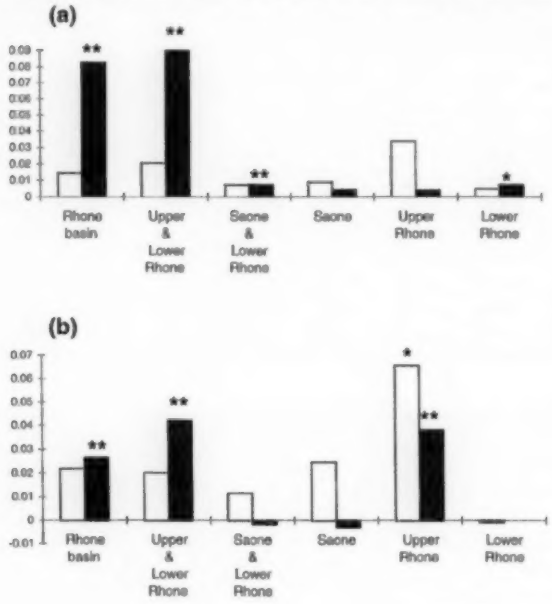
Fig. 2. Rhone River F statistics (open bars, F_{is}; solid bars, F_{st}) using different spatial scales for (*a*) chub and (*b*) roach populations.



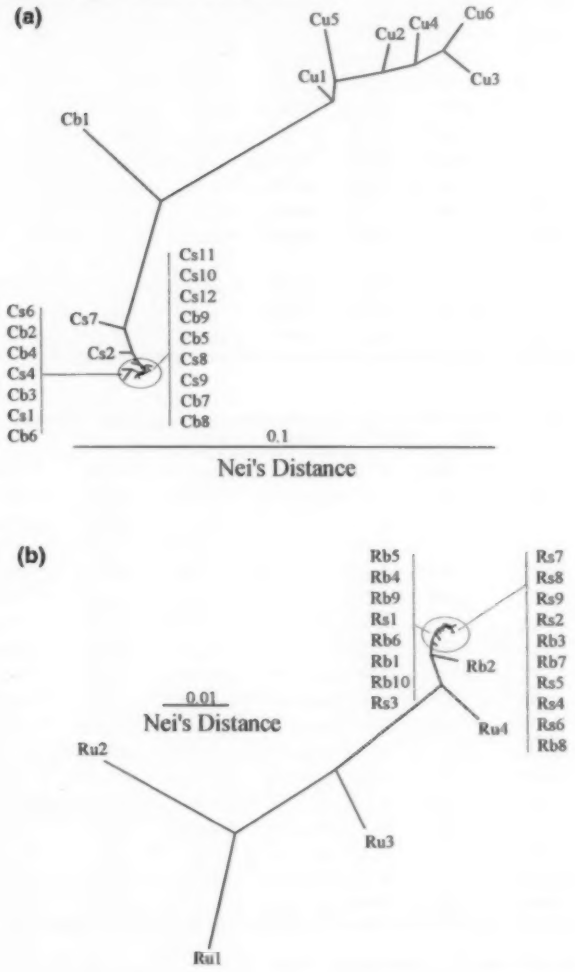
populations appeared more pronounced for chub (F_{st} = 0.083) than for roach (F_{st} = 0.026) (Fig. 2). Different spatial scales were then tested. The group of Upper Rhone and Lower Rhone populations displayed a significant differentiation (chub: F_{st} = 0.089; roach: F_{st} = 0.042) compared with the weak differentiation observed for the group of Saone and Lower Rhone populations (chub: F_{st} = 0.007; roach: F_{st} = 0). Low values of F_{st} were observed within each of the three main subsystems for both species, with the exception of a more pronounced differentiation among the roach populations of the Upper Rhone (F_{st} = 0.038). The F_{is} values were not different from 0 ($p < 0.05$) (Fig. 2), except for the Upper Rhone roach populations where F_{is} was significant (F_{is} = 0.065, $p < 0.05$).

The neighbor-joining analysis of genetic distances highlighted the marked clustering of Upper Rhone samples in comparison with a mixture of Saone and Lower Rhone samples for chub and roach (Fig. 3) and thus confirmed the general trend observed with F statistics. For chub and roach, a Middle Rhone station (Cb1 and Rb2, respectively) downstream the city of Lyon separated Upper Rhone from Saone and Lower Rhone populations.

Isolation by distance

Over geographic distances extending from 70 to 100 km, a significant multilocus positive correlation between genetic and geographic distances was observed for the chub along the Grosne–Saone continuum. The other river continuums displayed a significant isolation by distance for only one locus, *PGM-2* (Table 3). No significant relationship between genetic distance and geographic distance was detected for the roach, whichever river continuum was considered (Table 3).

Fig. 3. Neighbor-joining analysis of genetic distances for (*a*) chub and (*b*) roach populations.



Discussion

Population genetic variation

Chub and roach displayed a large niche width and large populations in the Rhone system (Carrel and Rivier 1996) and therefore exhibited relatively high values of heterozygosity similar to the mean value obtained for freshwater fish (mean $H_o \approx 0.043$; Gyllensten 1985; Ward et al. 1994).

Among European rivers, chub polymorphism and heterozygosity were significantly higher in the Rhine, Elbe, and Danube ($P > 95 \approx 21\%$; $H_e \approx 0.087$; Hänfling and Brandl 1998) than in the Rhone. Levels of heterozygosity showed a much wider range for chub in Greece (from 0.019 to 0.079), with the lowest degree of heterozygosity being observed in a small stream (Havrias) that sporadically dries up and was inhabited by a small population (Gollmann et al. 1996). Roach polymorphism and heterozygosity were similar in the Oder and Spree ($P > 95 \approx 18\%$; $H_e \approx 0.072$; Wolter 1998) but were

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Table 3. Correlation between genetic distance and geographic distance tested with the Mantel test.

Chub areas	Maximum distance (km)	Multilocus	*PGM-2*	*IDHP-1*	*EST-2*	*G3PDH*		
Saone	101.7	0.488*	ns	ns	ns	0.441*		
Lower Rhone (bypassed section)	76.9	ns	0.891**	ns	ns	ns		
Lower Rhone (diversion canal)	73.5	ns	0.851***	ns	ns	ns		
Roach areas			*AAT-2*	*G6DPH*	*GPI-1*	*GPI-2*	*PGM-2*	*MPI-2*
Saone	75.1	ns	ns	ns	ns	ns	ns	ns
Lower Rhone (bypassed section)	76.9	ns	ns	ns	ns	ns	ns	ns
Lower Rhone (diversion canal)	73.5	ns	ns	ns	ns	ns	ns	ns

Note: *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ns, nonsignificant.

significantly higher in the Danube ($P > 95 \approx 43\%$; $H_o \approx 0.2$; Baranyi et al. 1997). These comparisons must be considered carefully because the variability in polymorphism and heterozygosity between different rivers could be more associated with the number of studied loci than with various environments, perturbations, or historical processes.

The general trend in the distribution of chub and roach polymorphism and heterozygosity over the three main Rhone subsystems confirmed previous results (Bouvet et al. 1995). Lower polymorphism was detected for chub in the Saone than in the Lower and Upper Rhone. Heterozygosity varied in accordance with polymorphism for chub and displayed a higher value in the Upper Rhone. The slow-flowing Saone is characterized by silty and sandy bottoms; its environmental diversity is lower than that of the Upper Rhone with fast-flowing runs and cobble beds, dead arms, and adjacent lake (Pattée 1988). Chub genetic variability and environmental variability appear to be associated in the Rhone basin, as was pointed out for other fish (Shaw et al. 1994).

On a local scale, no detectable decrease in polymorphism or heterozygosity was observed for populations of chub and roach living in the heavily polluted sites of the Rhone's Chemical Corridor, as was demonstrated for different fish species living in contaminated rivers (Gillespie and Guttman 1993; Heithaus and Laushman 1997). The in vivo response of chub exposed to pollutants was assessed using two biomarkers, 7-ethoxyresorufin-*O*-deethylase induction and DNA single-strand breaks, and showed that 7-ethoxyresorufin-*O*-deethylase activity as well as DNA damage were found to be higher in the Rhone's Chemical Corridor compared with the reference station (Devaux et al. 1998). These results highlight that if the response to chemical stress at the cellular level is well marked for chub, the response at a higher level of biological organisation such as the population is more difficult to detect. Considering the classification of stream fishes based on ecological character and pollution tolerance (Heithaus and Laushman 1997), the risk of genetic erosion induced by pollution in chub and roach is probably low because these species are considered as generalists and pollution tolerant. In addition, chub and roach populations in polluted sites of the Lower Rhone remain large, and the risk of collapse and subsequent genetic drift is highly improbable. Extinction of certain individuals in the Chemical Corridor could be compensated by the drift of allochtonous fish coming from upstream and maintaining a high diversity.

An impact of hydroelectric schemes on genetic variation was detected. A slight decrease in polymorphism (chub and roach) and heterozygosity (chub) was shown below the diversion and the hydroelectric dams of Donzère Mondragon (erected 45 years ago), and we believe that this trend is biologically significant. Significant genetic divergence between populations localized upstream and downstream from the dams was detected; heterogeneity was mainly observed in the sector of the hydroelectric dam and lock for chub and in the sector of the diversion dam for roach. This genetic heterogeneity associated with dams may be linked to water quality and reduction of gene flow. Eurythermy is greatly marked for roach in contrast with chub, particularly in the warm outflows of the nuclear plant localized upstream from the hydroelectric lock and dam of Donzère Mondragon (Carrel and Rivier 1996). These results suggest that thermal stress is probably the main factor explaining the genetic heterogeneity for chub in the canal and that genetic heterogeneity for roach in the old channel is probably linked to other factors such as reduced gene flow. Furthermore, high infestation of cyprinids by *Bucephalus* in this sector of Donzère Mondragon (Pattée 1988) could also exert selective pressure on the populations.

The literature on the impact of dams on fish genetic variation has shown contrasting results. An increased heterozygosity was correlated with cold discharge from a hydroelectric dam (Zimmerman and Richmond 1981), whereas heterozygosity decreased downstream from a dam and reached a minimum value 30 km below (Smith et al. 1983). A decrease in heterozygosity was shown for chub populations below dams in the Aggitis and Ardas rivers (erected 30 years ago) in Greece (Gollmann et al. 1996), and the authors suggested that dams may interrupt gene flow; this low heterozygosity might also be due to fluctuations in water level creating bottlenecks and genetic drift.

Population differentiation

The F_{st} values for the whole Rhone Basin were relatively low, indicating appreciable gene flow between the populations, as usually observed for abundant and ubiquitous species (Tibbets and Dowling 1996). The majority of western European fish species have a Danubian origin. Connections between the Danube and Rhone were severed during the late Pliocene, and some fish persisted in the Saone and in southern Rhone tributaries during the late quaternary glaciations, the Upper Rhone being covered by ice (Pattée 1988; Bravard et al. 1992). Postglacial recolonization in the Rhone began 15 000 years ago and could explain this relatively low differenciation (Gyllensten 1985). Similar values of F_{st} were

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computed for chub in the Rhine, Elbe, and Danube (Hänfling and Brandl 1998) and for roach in the Danube (Baranyi et al. 1997).

The major structuring effect over the three Rhone subsystems observed for both chub and roach was the north–south genetic convergence among Saone and Lower Rhone populations. The *F* statistics and neighbor-joining analysis confirmed this general trend, which has been observed in chub using multiple correspondence analysis (Guinand et al. 1996). The intermediary position of Middle Rhone stations downstream from Lyon observed with neighbor-joining analysis (between Saone and Lower Rhone and the Upper Rhone) could indicate that the genetic characteristics of these populations are probably linked to similar gene flow from the Saone and Upper Rhone. The genetic distinctiveness of the Upper Rhone for both chub and roach could be the result of historical factors and (or) a selective pressure in a variable environment.

At present, the Upper Rhone fish assemblage is differentiated from that in the Lower Rhone, the discontinuity being detectable at the Saone confluence; a great similarity was observed between the fish fauna of the Saone and Lower Rhone (Changeux 1994). The hydroelectric schemes and particularly the creation of a succession of reservoirs since 1950 favored the development of lentic fish species in the Lower Rhone (Roux et al. 1989; Fruget 1992); roach have become dominant since 1960, and chub displayed no change in distribution but a growing biomass. In contrast, roach have been abundant in the Saone, probably for a long time. The distribution of benthic invertebrates in the Rhone system displayed the same trend (Berrahou 1993); the fauna of the Lower Saone and Lower Rhone were quite similar with a dominance of lentic pollution-tolerant species. Overall, the fish and invertebrate fauna of the Saone and Lower Rhone are becoming more similar with the increasing anthropogenic pressures leading to similar environmental conditions (hydraulic and probably thermal variables, water quality). Consequently, we suggest that selection is acting upon the Lower Rhone populations and might explain the genetic convergence between them and those of the Saone.

Within each Rhone subsystem, F_{st} for chub were generally low or null, which indicates a high level of gene flow between the populations. Roach exhibited a contrasting situation with a null F_{st} in the Saone and Lower Rhone and a significant F_{st} in the Upper Rhone. The Saone and probably the Lower Rhone represent a uniformly favorable environment for roach. Subpopulations are broadly distributed over these subsystems, and they exhibit a low differentiation. Environmental conditions are harsher for roach in the Upper Rhone because of high current flow in the main channel. Therefore, this lentic fish is concentrated in marginal habitats (side arms, lakes) leading to higher differentiation between populations (Bouvet et al. 1995). The spatial structure of the system markedly influenced the populational differentiation of roach.

The F_{is} values were nonsignificant for chub and roach, which suggests that the populations were probably numerous. Only the Upper Rhone populations of roach showed a deficiency of heterozygotes, suggesting a Wahlund effect (mixture of subpopulations differing in allele frequencies). This result is in agreement with the hypothesis of discrete populations exploiting marginal habitats.

The results of the Mantel test must be interpreted cautiously because of the limited number of sampling stations in the Saone and Lower Rhone. However, a trend of isolation by distance was observed for chub in the less perturbed system over 100 km, in contrast with the absence of a relationship between geographic distance and genetic distance for roach, whatever the system tested. The spatial dispersion of the individuals was therefore probably more limited for chub than for roach. The chub populations may interact following the stepping-stone model (Kimura and Weiss 1964). Chub is a litophil species and more ubiquitous than roach (Mann 1996) and it can spawn in the reservoirs on stony substrata (Kirka 1965) or migrate into tributaries with a precise reproductive homing (Le Louarn et al. 1997). The migration distances reported for this species are relatively limited, close to 10 km (Nicolas 1996; Wolter and Vilcinskas 1998). A high mobility linked to seasonal variation was clearly demonstrated in roach, and a precise reproductive homing was detected and was followed by a general dispersion of the postspawning adults (L'Abée-Lund and Vollestad 1985). The lack of isolation by distance for roach in the Rhone basin may reflect the low spatial structuration of fish in large rivers outside the spawning period.

In the heavily perturbed sector of the Lower Rhone, the correlation between genetic and geographic distances for chub was observed exclusively for one locus. In such a complex system, other factors affect dispersal rates and may make geographic distance a poor predictor of genetic differentiation. As seen before, the change in water quality and the possible reduced gene flow induced by dams could be the major factors affecting the populations.

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References

Agence de Bassin Rhône–Méditerranée–Corse. 1998. Qualité des cours d'eau. Synthèse 1995–1996. Rapport Agence de l'Eau Rhône–Méditerranée–Corse, Agence de l'Eau, Lyon.

Baranyi, C., Gollmann, G., and Bobin, M. 1997. Genetic and morphological variability in roach, *Rutilus rutilus*, from Austria. Hydrobiologia, **350**: 13–23.

Belkhir, K., Borsat, P., Goudet, J., and Bonhomme, F. 1996. GENETIX, logiciel sous Windows pour la génétique des populations. Laboratoire Génome et Populations, CNRS UPR 9060, Université de Montpellier II, Montpellier, France.

Berrahou, A. 1993. Répartition longitudinale des macroinvertébrés du Rhône français et sa relation avec les principaux affluents. Thèse de Doctorat, Université Claude Bernard Lyon I, France.

Bouvet, Y., Bobin, M., Maslin, J.L., and Pattée, E. 1995. The genetic structure of roach populations in two contrasted large rivers. Hydrobiologia, **303**: 229–234.

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Bravard, J.P., Roux, A.L., Amoros, C., and Reygrobellet, J.L. 1992. The Rhône River: a large alluvial temperate river. *In* The rivers handbooks. Hydrological and ecological principles. Vol. I. *Edited by* P. Calow and G.E. Petts. Blackwell Scientific Publications, Oxford, England. pp. 426–447.

Carrel, G., and Rivier, B. 1996. Distribution of three euryoecious cyprinids in the main channel of the lower River Rhône. Arch. Hydrobiol. Suppl. **113**: 363–374.

Changeux, T. 1994. Structure des peuplements de poissons à l'échelle du Bassin Rhodanien. Thèse de Doctorat, Université Claude Bernard Lyon I, France.

Coelho, M.M., Brito, R.M., Pacheco, T.R., Figueiredo, D., and Pires, A.M. 1995. Genetic variation and divergence of *Leuciscus pyrenaicus* and *L. carolitertii* (Pisces, Cyprinidae). J. Fish Biol. **47**(Suppl.): 243–258.

Devaux, A., Flammarion, P., Bernardon, V., Garric, J., and Monod, G. 1998. Monitoring of the chemical pollution of the River Rhône through measurement of DNA damage and cytochrome P4501A induction in chub (*Leuciscus cephalus*). Mar. Environ. Res. **46**(1–5): 257–262.

Felsenstein, J. 1993. PHYLIP (phylogeny inference package), version 3.5c. Distributed by the author, Department of Genetics, University of Washington, Seattle, Wash.

Fruget, J.F. 1992. Ecology of the lower Rhône after 200 years of human influence: a review. Regul. Rivers Res. Manage. **7**: 233–246.

Gillespie, R.B., and Guttman, S.I. 1993. Correlations between water quality and frequencies of allozyme genotypes in spotfin shiner (*Notropis spilopteris*) populations. Environ. Pollut. **81**: 147–150.

Gollmann, G., Bouvet, Y., Brito, R.M., Coelho, M.M., Collares-Pereira, M.J., Imsiridou, A., Karakousis, Y., Pattée, E., and Triantaphyllidis, C. 1996. Effects of river engineering on genetic structure of European fish populations. *In* International Conference on Fish Migration and Fish Bypass-Channel, 24–26 September 1996, Vienna, Austria. *Edited by* M. Jungwirth, S. Schmutz, and S. Weiss. Blackwell Science Ltd., Oxford, U.K.

Gollmann, G., Bouvet, Y., Karakousis, Y., and Triantaphyllidis, C. 1997. Genetic variability in *Chondrostoma* from Austrian, French and Greek rivers (Teleostei, Cyprinidae). Z. Zool. Syst. Evolutionsforsch. **35**: 165–169.

Guinand, B., Bouvet, Y., and Brohon, B. 1996. Spatial aspects of genetic differentiation of the European chub in the Rhône River basin. J. Fish Biol. **49**: 714–726.

Gyllensten, V. 1985. The genetic structure of fish: differences in the intraspecific distribution of biochemical genetic variation between marine, anadromous, and freshwater species. J. Fish Biol. **26**: 691–699.

Hänfling, B., and Brandl, R. 1998. Genetic and morphological variation in a common European cyprinid, *Leuciscus cephalus*, within and across central European drainages. J. Fish Biol. **52**: 706–715.

Heithaus, M.R., and Laushman, R.H. 1997. Genetic variation and conservation of stream fishes: influence of ecology, life history, and water quality. Can. J. Fish. Aquat. Sci. **54**: 1822–1836.

Kimura, M., and Weiss, G.H. 1964. The stepping stone model of population structure and the decrease of genetic correlation with distance. Genetics, **49**: 561–576.

Kirka, A. 1965. Age and growth of the chub *Leuciscus cephalus* (L.) from the Orava Reservoir and its drainage area. Zool. Listy, **14**: 235–250.

L'Abée-Lund, J.H., and Vollestad, A. 1985. Homing precision of roach *Rutilus rutilus* in Lake Arungen, Norway. Environ. Biol. Fishes, **13**: 235–239.

Le Louarn, H., Baglinière, J.L., Marchand, F., and Hamonet, J.M. 1997. Caractéristiques biologiques et écologiques du chevaine (*Leuciscus cephalus*) dans quelques rivières de la façade atlantique française. Inst. Natl. Rech. Agron. Bull. Sci. Tech. No. 29.

Mann, R.H.K. 1996. Environmental requirements of European non-salmonid fish in rivers. Hydrobiologia, **323**: 223–235.

McClenaghan, L.R., Smith, M.H., and Smith, M.W. 1985. Biochemical genetics of mosquitofish. IV. Changes of allele frequencies through time and space. Evolution, **39**: 451–460.

Mitton, J.B., and Lewis, W.M. 1989. Relationships between genetic variability and life-history features of bony fishes. Evolution, **43**: 1712–1723.

Nei, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics, **89**: 583–590.

Nicolas, Y. 1996. Le peuplement piscicole du Bas-Rhône: occupation d'un espace fluvial aménagé et typologie des sites de nursery et de refuge. Thèse de Doctorat, Université Claude Bernard Lyon I, France.

Pattée, E. 1988. Fish and their environment in large European river ecosystems, the Rhône. Sci. Eau, **7**: 35–74.

Pont, D., Allardi, J., Belliard, J., Boet, P., Carrel, G., Changeux, T., Oberdorff, T., Olivier, J.M., Persat, H., and Poizat, G. 1995. Stratégies démographiques des poissons des rivières françaises: premiers résultats. Bull. Fr. Pêche Piscic. **337/338/339**: 113–119.

Reynolds, J., Weir, B.S., and Cockerham, C.C. 1983. Estimation of the coancestry coefficient: basis for a short-term genetic distance. Genetics, **105**: 767–779.

Roux, A.L., Bravard, J.P., Amoros, C., and Pautou, G. 1989. Ecological changes of the French Upper Rhône River since 1750. *In* Historical change of large alluvial rivers: western Europe. *Edited by* G.E. Petts. John Wiley & Sons, London. pp. 323–350.

Shaklee, J.B., Allendorf, F.W., Morizot, D.C., and Whitt, G.S. 1990. Gene nomenclature for protein-coding loci in fish. Trans. Am. Fish. Soc. **119**: 2–15.

Shaw, P.W., Carvalho, G.R., Magurran, A.E., and Seghers, B.H. 1994. Factors affecting the distribution of genetic variability in the guppy, *Poecilia reticulata*. J. Fish Biol. **45**: 875–888.

Smith, M.W., Smith, M.H., and Chesser, R.K. 1983. Biochemical genetics of mosquitofish. I. Environmental correlates, and temporal and spatial heterogeneity of allele frequencies within a river drainage. Copeia, **1**: 182–193.

Tibbets, C.A., and Dowling, T.E. 1996. Effects of intrinsic and extrinsic factors on population fragmentation in three species of North American minnows (Teleostei: Cyprinidae). Evolution, **50**: 1280–1292.

Ward, R.D., Woodwark, M., and Skibinski, D.O.F. 1994. A comparison of genetic diversity levels in marine, freshwater, and anadromous fishes. J. Fish Biol. **44**: 213–232.

Weir, B.S., and Cockerham, C.C. 1984. Estimating *F*-statistics for the analysis of population structure. Evolution, **38**: 1358–1370.

Wolter, C. 1998. Estimation of gene flow between subpopulations of bream, *Abramis brama*, white bream, *Abramis bjoerkna*, roach, *Rutilus rutilus*, and rudd, *Scardinius erythrophthalmus*, within the River Spree basin. Ber. Inst. Gewässerökol. Binnenfisch. (Berl.), **5**: 71–78.

Wolter, C., and Vilcinskas, A. 1998. Effects of canalization on fish migrations in canals and regulated rivers. Pol. Arch. Hydrobiol. **45**: 91–101.

Young, A., Boyle, T., and Brown, T. 1996. The population genetic consequences of habitat fragmentation for plants. Trends Ecol. Evol. **11**: 413–418.

Zimmerman, E.G., and Richmond, M.C. 1981. Increased heterozygosity at the *Mdh-B* locus in fish inhabiting a rapidly fluctuating thermal environment. Trans. Am. Fish. Soc. **110**: 410–416.

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Fertilization biology of the abalone *Haliotis laevigata*: laboratory and field studies

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Abstract: A combination of laboratory and field experiments with the commercial abalone species *Haliotis laevigata* showed that fertilization may be a limiting factor in some exploited populations when distances separating spawning individuals are too large. The effects of gamete age, gamete concentration, and gamete contact time in the laboratory were used to model fertilization success in situ and compared with experimental fertilization rates in the field. Highest fertilization rates in vitro (80%) were found for sperm concentrations in the range of 1×10^4 to $1 \times 10^6 \cdot mL^{-1}$. Fertilization rates of 48 ± 1.7% (95% CI) were measured at separation distances of 2 m and dropped to 2.8 ± 0.7% (95% CI) at 16 m downstream, agreeing closely with rates predicted by the model. Recruitment failures reported for South Australian populations of *H. laevigata* have occurred when densities fell below ~0.3 animals$\cdot m^{-2}$, or mean nearest-neighbor distances between 1 and 2 m. This density corresponds well to critical nearest-neighbor distances for fertilization success. Stocks at higher densities are predicted to have higher fertilization rates (~90%) such that fertilization success is not a factor limiting recruitment.

Résumé : La combinaison d'expériences de laboratoire et de terrain sur l'ormeau commercialement exploité *Haliotis laevigata* a montré que la fécondation peut être un facteur limitant chez certaines populations exploitées quand la distance qui sépare les individus est trop grande au moment de la reproduction. Les effets de l'âge des gamètes, de leur concentration et du temps de contact entre elles en laboratoire ont servi à modéliser le succès de fécondation in situ et ont été comparés aux taux expérimentaux de fécondation sur le terrain. Les taux de fécondation in vitro les plus élevés (80 %) correspondaient à des concentrations de sperme de l'ordre de $1 \times 10^4 - 1 \times 10^6$ mL^{-1}. Des taux de fécondation de 48 ± 1,7 % (95 % CI) ont été mesurés lorsque les distances séparant les individus étaient de 2 m; ils tombaient à 2,8 ± 0,7 % (95 % CI) à 16 m en aval, ce qui correspond de près aux prédictions du modèle. Les échecs du recrutement signalés pour des populations de *H. laevigata* d'Australie méridionale se sont produits lorsque les densités sont tombées au-dessous de ~ 0,3 animaux$\cdot m^{-2}$, ou avec des distances moyennes du voisin le plus proche situées entre 1 et 2 m. Cette densité concorde avec les distances du voisin le plus proche qui sont critiques pour le succès de la fécondation. On prévoit que les stocks à forte densité doivent connaître un taux plus élevé de fécondation (environ 90 %), de sorte que le succès de fécondation n'est pas un facteur qui limite le recrutement.

[Traduit par la Rédaction]

Introduction

Since the early part of this century, it has been suggested that reproductive failure in commercially harvested free-spawning marine species might be a cause of stock decline or recruitment failure (reviewed in Levitan 1995). Population densities might be lowered to such an extent that by the time sperm reached the vicinity of an egg, the probability of fertilization would be low or negligible. This limitation on reproductive success is known as the Allee effect (Allee 1931). More recently, the need to explicitly incorporate Allee effects in the management of marine invertebrate resources has been highlighted for several groups, including abalone (Breen and Adkins 1980; Shepherd 1986; Shepherd and Brown 1993), sea urchins (Quinn et al. 1993), scallops (Caddy1988), and giant clams (Munro 1993). However, the empirical basis for putting such management strategies into effect remains poorly developed.

In parallel to the development of these ideas, interest directed towards fundamental understanding of how fertilization success in marine invertebrates is influenced by the interactions of organisms and their environment has also been growing. Rekindling of interest in this topic was sparked by field experiments with the sea urchin *Strongylocentrotus purpuratus* (Pennington 1985) that demonstrated that, even for individuals separated by as little as 1 m, fertilization rates could be <10%. Other field studies on sea urchins (Levitan 1991) as well as numerical modeling studies (Denny and Shibata 1989) have confirmed this trend. Measurements of fertilization rates in natural spawnings subsequently showed that, despite rapid dilution of sperm, fertilization rates of a wide range of marine invertebrates could be high (>90%) but also highly variable (Brazeau and Lasker 1990; Yund 1990; Babcock et al. 1992; Sewell and Levitan 1992). This variability is consistent with suggestions that at low densities, reproductive success can be a limiting factor in populations of marine invertebrates.

Attempts have been made to model the role of Allee effects on populations of commercially exploited marine in-

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vertebrates in order to either better understand the history of a stock (*Haliotis laevigata*, Shepherd and Partington 1995) or formulate management strategies (*Strongylocentrotus franciscanus*, Quinn et al. 1993). These have calculated that reproductive success is highly sensitive to variations in density and that management must consider Allee effects in order to be effective. Some long-lived species may, however, be relatively insensitive to these effects (e.g., *S. franciscanus*, Ebert 1998).

In the case of *H. laevigata*, Shepherd and Partington (1995) used a long-term (11-year) data set comparing stock density and biomass with recruitment in an isolated population to demonstrate a dependence of recruitment on stock density and biomass. By applying a theoretical model of zygote production (based on data from echinoderms), they showed how a rapid decline in zygote production could occur at low adult density, which might explain recruitment patterns observed in the population.

Empirical studies of the fertilization kinetics of mollusks such as abalone have lagged behind those of echinoderms, which have been used most frequently as experimental models of gamete interactions and diffusion in the sea (Levitan et al. 1991; Babcock et al. 1994; Benzie and Dixon 1994; Levitan and Young 1995). Here, we present the first measurements of fertilization of the abalone *H. laevigata* in the field. The overall aim of this study was to obtain biological information that would strengthen our understanding of how density influences reproductive success and the maintenance of *H. laevigata* populations. We obtained a variety of information on fertilization kinetics, including the effects of sperm dilution, gamete age, and gamete contact time, as well as experimental in situ fertilization rates. These data were compared with published information on population trends in *H. laevigata* populations from South Australia.

Methods

Spawning induction

Field work was carried out from the RV *Ngerin* immediately prior to the predicted spawning season (Rodda et al. 1997) of *H. laevigata* in the Western Spencer Gulf region during the periods 2–10 November 1995 and 4–12 November 1996. During these times, it was anticipated that difficulties associated with spawning induction would be low. Repeated initial attempts to induce spawning using the H_2O_2 procedures of Tong and Moss (1992), temperature shock (3–5°C above ambient, 16 ± 0.5°C for 2–3 h), and desiccation (0.5 h on deck) resulted in only a few desultory spawnings that failed to produce viable gametes. During the period while these spawning attempts were being made, a number of animals held in tubs supplied with running seawater spawned spontaneously, generally after being held with 20–30 other animals for a period of 4 h or more. Consequently, we decided to use aggregation to obtain gametes for our experiments.

To induce spawning in *H. laevigata*, we collected 20–30 animals of each sex and placed them into 300-L tubs with flow-through seawater. The sex of each animal was determined by external examination (male gonad yellow, female gonad green) and sorting on deck; thus, the animals were out of water for a period of up to 15 min. Groups of male and female abalone were then placed in separate tubs. Prior to the anticipated spawning, the flow-through seawater system was replaced with independent recirculating systems to avoid possible gamete cross-contamination. As soon as spawning was noticed (between 5 and 12 h after collection), all animals were rinsed and transferred to individual 10-L buckets containing 2–4 L of seawater. Males, in particular, were placed in as small a volume as practical to obtain concentrated gametes and minimize respiratory dilution effects (Chia and Bickell 1983) on sperm viability. The transfers allowed us to obtain gametes from separate individuals, and handling did not appear to affect the spawning process when done after spawning had commenced.

To more formally assess the effect of aggregation on spawning behaviors, we set up two experiments. Animals collected at the same site and time were placed in two separate treatments, with one group being held in tubs and an equivalent number of individuals isolated in 10-L buckets. The number of spawning animals in each treatment was noted after 10 h. This procedure was done on two occasions, once during the day with sexes separate and the other overnight with both sexes combined.

Gamete release rates

Once animals were transferred from spawning tubs to individual buckets, they were allowed to spawn for 3 h. Their lengths were then measured to the nearest millimetre, and they were returned to the sea. The volume of seawater remaining in the bucket was then measured and a subsample of the gamete suspension was taken after mixing to ensure that gametes were distributed evenly during sampling. Sperm concentration was determined by five replicate haemocytometer counts and egg concentration was determined from three replicate counts of 0.25-mL aliquots.

Sperm dilution pilot study

A concentrated sperm suspension was reduced nine times by serial 10-fold dilutions to produce a range of sperm concentrations. In total, there were 11 treatments including the dilutions and a control treatment to which no sperm had been added. Immediately after the dilutions were made, 9 mL of each suspension and 1 mL of concentrated egg suspension (~$1 \times 10^3 \cdot mL^{-1}$) was placed in 20-mL glass scintillation vials. Three replicate vials were prepared at each dilution. Three vials containing only seawater and eggs were used as controls. After 3 h, the fertilization rate was scored as the proportion of eggs that were undergoing cleavage. Given the low level of variability among replicate vials in the pilot study, only two vials were used in the subsequent gamete age and contact time experiments.

Sperm–egg contact time and sperm dilution

The effects of sperm–egg contact time and sperm dilution were assessed in a series of four replicate experiments in which eggs were immersed in sperm suspensions at a range of dilutions and then removed and rinsed at predetermined time intervals (7, 15, 30, 60, 120, 240, 600, 1200, and 2400 s). Since a variety of empirical and modeling studies have indicated that egg concentration and sperm to egg ratio are much less important than sperm concentration (Lillie 1915; Vogel et al. 1982; Levitan et al. 1991), we held egg concentration constant at $3.6 \times 10^3 \cdot mL^{-1}$. The method we used was similar to that described by Levitan et al. (1991). Plastic tubes with one end covered by 64-mm-mesh screen were placed into petrie dishes that held sperm suspensions at seven 10-fold serial dilutions. One-millilitre aliquots of egg suspension were then pipetted into the partially immersed tubes. After the prescribed contact time, the immersed eggs were removed from the sperm suspension, rinsed by irrigating them with sperm-free seawater, and placed in individual dishes of sperm-free water. The proportion of fertilized eggs was scored after 3 h.

Sperm age experiment

Concentrated sperm suspensions were serially diluted and combined with eggs in 20-mL vials (9 mL of sperm and 1 mL of eggs) as described for the pilot study. The first trial was completed within 15 min of diluting the concentrated gametes. Subsequently, gametes were combined at intervals of 30, 45, 60, 105, 120, 180,

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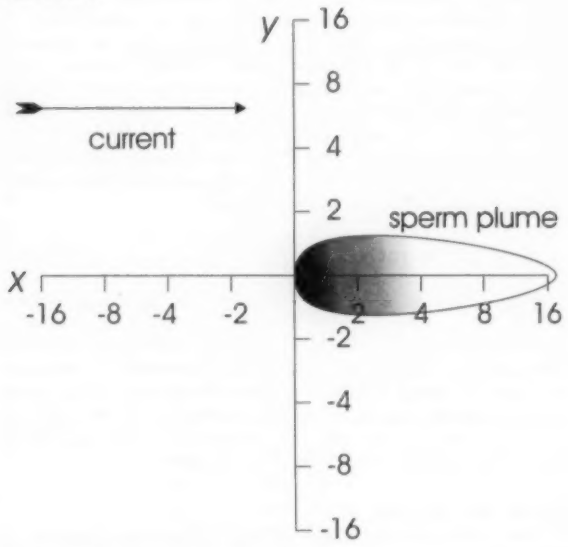
Fig. 1. Configuration of sampling sites for in situ fertilization experiments. The *x*-axis is aligned in the direction of current flow, with the point of sperm release at the origin of the *x*- and *y*-axes (0,0).



240, and 300 min after initial dilution at each of seven sperm concentrations. Fertilization was scored after 3 h.

Egg age experiment

Freshly spawned eggs from a single female (1-mL aliquot) were combined with 9 mL of sperm at two dilutions (2.4×10^5 and 1×10^4 sperm·mL^{-1}), as previously described. The sperm suspension used in the experiment was obtained in a highly concentrated form (2.4×10^{10}·mL^{-1}) by expressing sperm from a spawning male. Since sperm can only be expressed from animals that have already commenced the spawning process, this treatment did not affect gamete viability. These sperm were refrigerated at 5°C and reduced to the appropriate dilution immediately before each egg age treatment in order to reduce or eliminate any effects of sperm aging. At the time of the final egg aging treatment, after 7 h, freshly spawned eggs from another female were fertilized with the same sperm in order to assess any changes in sperm viability over the time of the experiment.

In situ assessment of fertilization rates

Gamete handling

We used gametes from animals that had spawned in buckets onboard ship as a means of simulating spawnings. Individual variability in the precise timing of gamete release did not permit us to use the animals themselves for field experiments. The way in which gametes were handled differed for male and female abalone. The concentration of each sperm suspension was determined by haemocytometer counts and adjusted to ~1×10^8·mL^{-1}. A plastic intravenous drip bag was then filled with a measured volume (~1 L) of this suspension. Plastic tubing terminating in a disposable pipette tip was attached to the bag and temporarily sealed by clamping the hose. The bag was then placed between two plastic slates loosely held together by rubber bands. Once underwater, sperm could be released at a controlled rate by using a 2-kg lead weight to apply pressure to the upper slate. The rate at which the contents were released was controlled by means of adjusting the clamp and trimming the aperture size of the pipette tip. Precise times of release start and finish and initial and final bag volumes were recorded in order to establish the exact sperm release rates for each experiment. The rates were designed to correspond to the sperm release rates measured from abalone spawning onboard ship (~5×10^7·s^{-1}) and varied between 1×10^7 and 5×10^7·s^{-1}.

As soon as a sufficient number of eggs were available for the experiment, they were collected by pipette from the bottom of buckets containing the female abalone. About 10 000 eggs and 300 mL of seawater were then dispensed into each of 12 individual sample cartridges of a submersible sampling pump (Mundy et al. 1994). Each numbered sample chamber consisted of an inner filter cartridge constructed of perspex and plankton mesh (32 µm). These cartridges, which contained the eggs, were each sealed within outer chambers so that no water could enter except when water was pumped through them during the act of sampling. Once charged with eggs, the sampling apparatus and the bag of concentrated sperm suspension were taken directly to the sampling site, and sampling commenced within 30 min of the eggs being spawned. The short interval between sampling and spawning and the use of concentrated sperm in the dispensing bag were designed to minimize gamete age artifacts.

Field sampling

Field experiments were carried out in depths between 4 and 10 m in areas typical of *H. laevigata* habitat. The basic principle underlying the field sampling was to simulate the natural release of gametes by spawning animals and to obtain samples of seawater that would allow us to assess how gamete dispersal and fertilization rates varied with distance from the point of sperm release. Sampling was oriented relative to the direction of water flow, since currents are likely to be a key factor affecting the dispersal of gametes. Current direction at the time of sampling was determined either using a small dye release or by tracking particles suspended in the water column. A tape measure was run out parallel to the current (*x*-axis) for distances of up to 16 m. A second tape was laid out at right angles to this (*y*-axis) to form a pair of orthogonal axes (Fig. 1). Using these axes the location of any sampling point could be referred to by a grid reference. Current speed was then estimated by timing the movement of particles over a predetermined distance along the tape.

Once the axes were established the bag containing sperm was placed at the intersection of the tapes and sperm release was started, simulating spawning. After allowing 5–10 min for the sperm plume to develop, samples were taken from a series of points at varying distances downstream from the point of sperm release. For each sample, ~2 L of seawater was pumped through one of the 12 numbered filter cartridges containing fresh eggs, so that the eggs were exposed to the sperm concentration that prevailed at each sampling point at the time of collection. This volume was intended to allow about seven times the original volume in the cartridge to be displaced during sampling. Samples were taken at distances of 16, 8, 4, 2, 1, and 0 m downstream from the spawning male and at a height of 0.1 m above the substratum. Sampling always started at the downstream end of the tape (most distant from the point of sperm release) and proceeded upstream. A fresh cartridge was used at each sampling point. A final sample was taken upstream from the male (–8 m) as a control for any sperm contamination. In addition to these samples taken directly downstream from the sperm release point (e.g., $x = 8$, $y = 0$; $x = 4$, $y = 0$, etc.), a subset of samples were taken at distances of either 2 or 4 m perpendicular to the downstream axis (e.g., $x = 8$, $y = 2$; $x = 4$, $y = 2$, etc). If no current was detectable, sampling was done symmetrically along each axis at distances increasingly closer to the point of sperm release (4, 2, 1, and 0 m) before a final control sample taken ~30 m from the point of sperm release. Eggs were extracted from the chambers after sampling and allowed to develop for a further 2 h before being scored for the proportion of eggs fertilized. Fertil-

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Table 1. Spawning induction of *H. laevigata*.

	4 November 1996, animals collected 18:30		9 November 1996, animals collected 10:00		
Behavior	Male/female tubs	Buckets	Male tubs	Female tubs	Buckets
Spawned	4 males (05:30–06:15)	0	10 (17:00)	7 (17:30)	0
	2 females (06:30–06:35)	0			
Did not spawn	15	15	4	9	10

Note: Times in parentheses indicate commencement or duration of observed spawning.

ization was assessed based on the presence–absence of initial cell cleavages.

Fertilization and sperm diffusion modeling

Vogel et al. (1982) developed a numerical model (eq. 1) to allow them to predict fertilization rate in conditions where several factors can vary simultaneously. Their fertilization kinetics model (the Vogel–Czihak–Chang–Wolf (VCCW) model), which relates fertilization rate (F) to gamete concentrations, sperm half-life (τ), and two fertilization rate constants (β, β_0), was used here to obtain estimates for *H. laevigata* fertilization rate constants. It is critical to obtain values for these parameters, since predictions of fertilization rates in the field based on gamete diffusion (e.g., Denny and Shibata 1989) are sensitive to these rate constants (Levitan et al. 1991). The model is

$$(1) \qquad F = 1 - \exp\left[-\frac{\beta}{\beta_0}\frac{S_0}{E_0}(1 - e^{-\beta_0 E_0 \tau})\right]$$

where F is fertilization rate, β is the rate fertilization constant based on sperm–egg contact and egg fertilizability, β_0 is the rate fertilization constant based on egg cross section and sperm speed, E_0 is egg concentration (per microlitre), S_0 is sperm concentration (per microlitre), and τ is sperm half-life (seconds).

The sperm–egg contact time and sperm dilution experiments above all used fresh gametes, and we fitted fertilization data from these to the VCCW model (eq. 1). We substituted t (sperm-egg contact time) for τ (sperm half-life) where t exceeded τ (Vogel et al. 1982, eq. 13), since values t of higher than τ would overestimate sperm viability (Levitan et al. 1991). Values of τ were estimated using data from the sperm age and sperm dilution experiments where, for each concentration, the slope of the relationship between fertilization rate and sperm age was used to calculate the sperm half-life (the time at which the fertilization rate was 50% of the initial value). These sperm half-lives were plotted against sperm concentration and a linear regression was used to allow us to predict sperm half-life at any sperm concentration. We used a Marquardt iterative method of nonlinear regression (Jandel Sigma Plot™) to determine the best fit for values of β and β_0 in the VCCW equation for our values of t, τ, sperm concentration, and fertilization rate. The model does not take polyspermy into account; therefore, dilutions 0 and 1 were not used in the fitting of the curve.

A numerical model of sperm diffusion (Babcock et al. 1994) was combined with the model for fertilization success and used to predict fertilization rates. Parameters in the diffusion model (eq. 2) were defined as follows: x, y, and z, longitudinal, horizontal, and vertical axes oriented according to current flow (see Fig. 1; these values define the sampling point); S, sperm concentration (per cubic metre); Q_s: sperm release rate (per second); $\bar{U}$, mean current velocity (metres per second), σ_y and σ_z, standard deviation of diffusion along the y- and z-axis; h, distance above the substratum at which sperm are released; d, depth of water. The model is

$$(2) \qquad S(x,y,z) = \left(\frac{Q_s}{2\pi U \sigma_y \sigma_z}\right)\left\{\exp\left[-y^2/2\sigma_y^2 - \left(\frac{(z-h)^2}{2\sigma_z^2}\right)\right] + \exp\left[-y^2/2\sigma_y^2 - \left(\frac{(z+h)^2}{2\sigma_z^2}\right)\right] + \exp\left[-y^2/2\sigma_y^2 - \left(\frac{2(d-z-h)^2}{2\sigma_z^2}\right)\right]\right\}$$

The model for fertilization rate of eggs and sperm in a closed container (eq. 3) was used to estimate fertilization rates based on predicted sperm concentrations (Vogel et al. 1982; Denny and Shibata 1989). The closed-container model is directly analogous to the containers in the submersible plankton pump and its use avoids potential concerns raised over containment artifacts (cf. Levitan 1995) in the plankton pump apparatus. Fertilization rate can then be estimated as

$$(3) \qquad F = 1 - \exp(-\theta S_t)$$

where θ (Denny and Shibata 1989) is the fertilization rate constant for eggs and sperm in a closed container.

Spatial distribution

Commercially fished populations of *H. laevigata* at Cathedral Rock and East Taylor's Island were sampled just before spawning during early November 1995 and 1996 in order to obtain measures of density and aggregation. The spatial statistics obtained should therefore represent those present in a spawning population with reasonable accuracy. Forty-nine quadrats (2 × 2 m) were haphazardly deployed in areas of suitable habitat at depths of 10–15 m. Density of mature *H. laevigata* (>100 mm shell length) was recorded as well as nearest-neighbor distances measured from the center of each animal. Data on the level of aggregation in each population were expressed in terms of the nearest-neighbor aggregation index R (Clark and Evans 1954).

Based on these data and published measures of density and aggregation during spawning season from two other South Australian populations (Waterloo Bay and West Island, Shepherd 1986), male–female nearest-neighbor distances were calculated from equations in Clarke and Evans (1954) assuming populations with 1:1 sex ratios. For the Waterloo Bay and West Island populations the densities used as the basis for nearest-neighbor densities in the table were those recorded at the times when recruitment to these populations failed (Shepherd and Brown 1993; Shepherd and Partington 1995).

Results

Spawning induction

A total of 76 individuals were used in spawning induction trials, 51 in the tubs and 25 in the buckets. All of the 23 animals in which spawning was observed were from the tubs

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Fig. 2. Total gamete output of *H. laevigata* per spawn for males (○) and females (●) over a 3-h period. Data are regressions ± 95% CI).

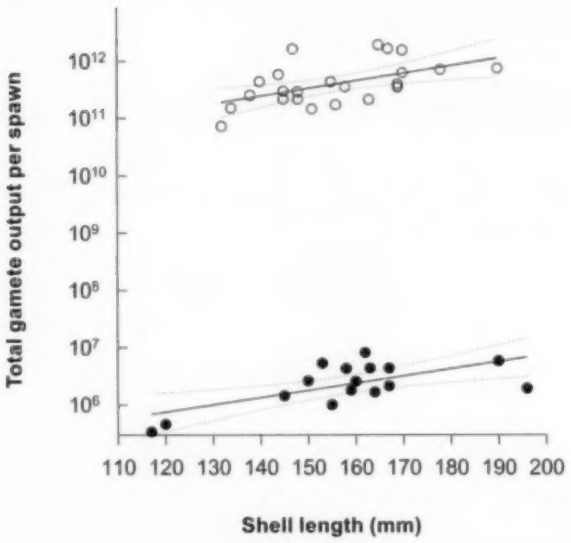
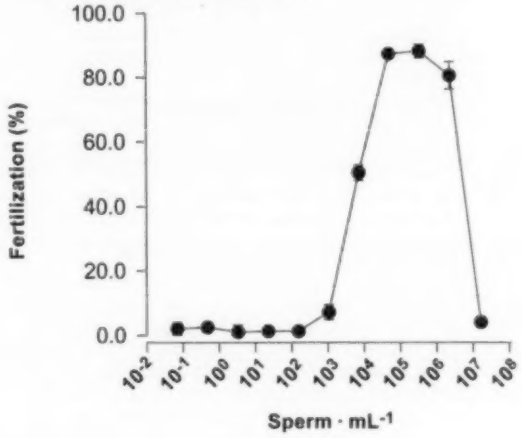


Fig. 3. Fertilization rate and sperm concentration for *H. laevigata*. Data are means ± 95% CI (some confidence intervals are obscured by the symbols).



(Table 1), and none of the animals isolated in buckets showed any sign of spawning, a highly significant difference ($\chi^2 = 16.25$, df = 1, $p < 0.05$). The technique worked during both night and day and when sexes were separated as well as when males and females were held in the same tubs. Observations from these experiments and from the numerous spawning inductions during the course of the study indicated that animals collected in the morning and held in tubs on deck generally spawned after 5–7 h, while those collected in the late afternoon spawned after 8–12 h. The temperature difference between these two treatments was up to 4°C (14.5–18.5°C).

Gamete release rates

The number of sperm released by 24 male *H. laevigata* over a 3-h period ranged from 7.1×10^{10} to 1.9×10^{12}, averaging $5.7 \times 10^{11} \pm 5.4 \times 10^{11}$ SD. Data obtained from 16 females indicated that the number of eggs released ranged from 3.4×10^5 to 8.2×10^6, averaging $3.0 \times 10^6 \pm 2.2 \times 10^6$ SD. The size ranges of animals used for the measurements varied appreciably (males, 132–190 mm; females, 117–196 mm); however, relationships between shell length and total gamete output (Fig. 2) were weak and not significant (log-transformed data, least-squares linear regression; males: $r^2 = 0.44$, df = 1, $F = 3.55$, $p = 0.081$; females: $r^2 = 0.28$, df = 1, $F = 3.67$, $p = 0.068$). Mean sperm release rate, as calculated over a 3-h period, was $5.3 \times 10^7 \pm 4.9 \times 10^7 \cdot s^{-1}$.

Sperm dilution pilot study

High levels of egg cleavage were observed only within a relatively narrow range of sperm concentrations, between 1×10^4 and $1 \times 10^6 \cdot mL^{-1}$ (Fig. 3). At concentrations lower than this, fertilization rate dropped rapidly due to low numbers of sperm, while at higher concentrations the level of successful fertilization, as assessed by the proportion of cleaving eggs, also dropped rapidly to low levels. The observed drop in cleavages at high concentrations was due to polyspermy and interference with normal cell division processes by supernumerary sperm or lysis of the egg membrane at the highest concentration. Dilution 0 was set in the order of 1×10^7 sperm·mL^{-1} for the remaining experiments. Since no fertilizations were recorded at the lower end of the dilution series (i.e., $<1 \times 10^3$ sperm·mL^{-1}), subsequent experiments were restricted to the upper end of the range of concentrations used.

Egg age

Initial fertilization rates of 79 and 58% for eggs mixed within 1 h of spawning were within the ranges expected for the concentrations used (1×10^4 and 1×10^5 sperm·mL^{-1}, Figs. 3 and 4). Both egg age and sperm concentration had significant effects on fertilization rate (data presented in Fig. 4, arcsine transformed prior to analysis, ANCOVA, $p < 0.0001$ for the effects of both gamete age and concentration). After 2 h, fertilization rates had not dropped significantly (Fig. 4), but by 3 h, some reductions in fertilization rates were apparent. For the first 3 h, fertilization rates could not be statistically separated at the 0.05 level (pairwise matrix of p values using corrected least-squares means), and these values were not significantly different from those of the fresh egg controls (Fig. 4). Fertilization rates continued to drop until the experiments were terminated after 7 h. At that time, the fertilization rates were 42% for eggs in the higher sperm concentrations but only 15% in the more dilute sperm. As controls for any sperm age effects, freshly spawned eggs were mixed with sperm at each dilution at that time. These eggs displayed fertilization rates almost identical to the initial values (79 and 76%) for eggs at the start of the aging experiment, indicating that the sperm used had retained a high level of activity throughout the experiment.

Sperm age and sperm dilution

Both sperm age and sperm dilution affected fertilization rate (Fig. 5). Statistical analysis of arcsine-transformed data

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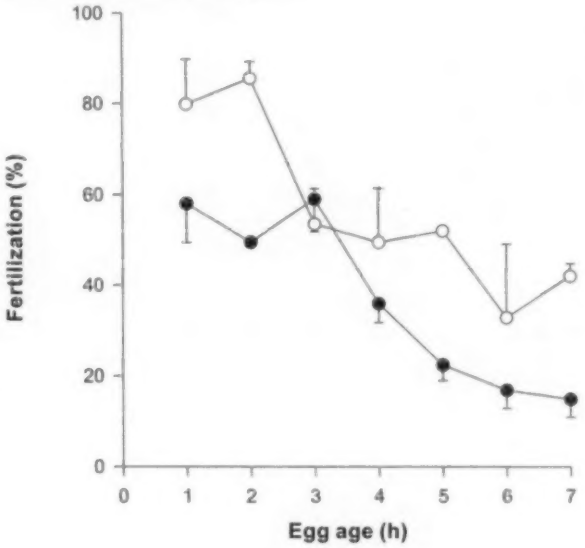
Fig. 4. Fertilization rate and egg age for *H. laevigata* showing the effects of egg age on fertilization rate at two sperm concentrations, $1 \times 10^5 \cdot mL^{-1}$ (○) and $1 \times 10^4 \cdot mL^{-1}$ (●). Data are means ± 95% CI (some confidence intervals are obscured by the symbols). Controls for sperm viability conducted with fresh eggs at sperm concentrations of 1×10^5 and $1 \times 10^4 \cdot mL^{-1}$ after 7 h gave 79 and 76% fertilization, respectively. Regressions: 1×10^5 sperm$\cdot mL^{-1}$, $y = -0.078x + 0.86$, $r^2 = 0.77$; 1×10^4 sperm$\cdot mL^{-1}$, $y = -0.083x + 0.69$, $r^2 = 0.87$. A posteriori pairwise comparisons indicated that for eggs 1–3 h old at 1×10^5 sperm$\cdot mL^{-1}$, fertilization rates were not significantly different from those of controls using fresh eggs. At 1×10^4 sperm$\cdot mL^{-1}$, fertilization rates for fresh eggs differed significantly only from those for 6- and 7-h-old eggs.



from two replicate experiments indicated that both factors were highly significant (ANCOVA, $p < 0.0001$ for both sperm age and sperm concentration). At high concentrations, fertilization rates were low, particularly for dilution 1, and increased slightly over time (Fig. 5). More dilute sperm suspensions (dilutions 2 and 3) produced the highest fertilization rates up to an age of 60 min, and beyond 120 min, aging effects rapidly reduced fertilization rates. At still lower dilutions, fertilization rates were low or negligible and aging effects were manifested more quickly (dilutions 4 and 5).

Sperm–egg contact time and sperm dilution

There was a highly significant influence of sperm concentration on fertilization rate (Fig. 6), but contact time was not significant (ANCOVA, sperm concentration: $p = 0.0001$; sperm–egg contact time: $p = 0.84$, data arcsine transformed prior to analysis). The lack of a significant effect for contact time was probably due to contrasting trends in fertilization rate with increasing contact times at high and low concentrations, although variability in the data no doubt also played a role. Individual regression analyses for each concentration indicated that slopes differed significantly from zero for both dilution 1 (negative) and dilution 4 (positive), as would be expected if polyspermy were a factor at high sperm concentrations and sperm limitation at low concentrations. At the extremes of high and low concentrations, gamete density effects appeared to swamp any effects that might occur due to contact time, while at intermediate densities, any trends were obscured by variability in the data. Apparently, most fertilization took place over a relatively short period of time.

In situ fertilization experiments

In total, 18 experiments were carried out under conditions with measurable net current flow and four where no discernable or consistent flow field was present. Fertilization rates at the point of sperm release averaged 87.6 ± 5.5% in the field, but dropped rapidly, reaching only 48 ± 1.7% by 2 m directly downstream. At 16 m downstream, fertilization rates averaged only 2.8 ± 0.7% (Fig. 7). Sampling sites offset by 2 and 4 m from the direct downstream axis produced progressively lower rates of fertilization that were consistent with the gradual lateral spread of the sperm plume along the *y*-axis (Fig. 7). Samples during conditions of no net current flow were restricted to distances of 4 m or less, but within this range of distances, fertilization rates proved to be roughly similar to those in the experiments with measurable currents. In these experiments, fertilization rates at the point of sperm release averaged 97 ± 2.8%, slightly higher than those when currents were flowing, but at 2 m distance, rates were 53 ± 28.6%, equivalent to the results in higher flow conditions (Fig. 7). At 4 m distance, fertilization rates were slightly lower without currents (16.9 ± 14.9%) than they were when currents were flowing (26.5 ± 10.5%).

Fertilization kinetics model

Sperm half-life ranged from 56 min at sperm concentrations $<1 \times 10^2 \cdot mL^{-1}$ up to >400 min at concentrations of $1 \times 10^6 \cdot mL^{-1}$ (Fig. 8). The value for the rate constant of fertilization, β, determined from the sperm age and sperm dilution experiments was 3.35×10^{-5}, while the sperm egg encounter rate constant, β_0, was 2.94×10^{-3}. The ratio of β/β_0 was 0.0108. This ratio was very similar to that of *Paracentrotus lividus* (Vogel et al. 1982); however, the actual values of the rate constants were higher and of the same order as those of *S. franciscanus* (Levitan et al. 1991). When regressed against the mean data from sperm–egg contact time and sperm dilution experiments, the VCCW predictions produced an r^2 value of 0.73.

Sperm diffusion model

Most of the model components were determined empirically; however, some parameters were obtained from the literature. The value for friction velocity (U^*) was set at 0.05 of $\bar{U}$, based on unpublished acoustic Doppler velocimeter data from a different site of similar depth and bottom profile (P. Osborne, Geography Department, University of Auckland, personal communication). Mean currents during the study were 0.055 $m \cdot s^{-1}$, and for the purposes of the model, average water depth was set at 5 m and release height at 0.1 m. Sperm release rates were set at $5 \times 10^7 \cdot s^{-1}$.

The fertilization rate constant θ used here corresponds to β of Vogel et al. (1982, eq. 7), which we have calculated as 3.35×10^{-5} $mm^3 \cdot s^{-1}$ (note that this must be scaled up to cubic metres for use in the model). The diffusion coefficients

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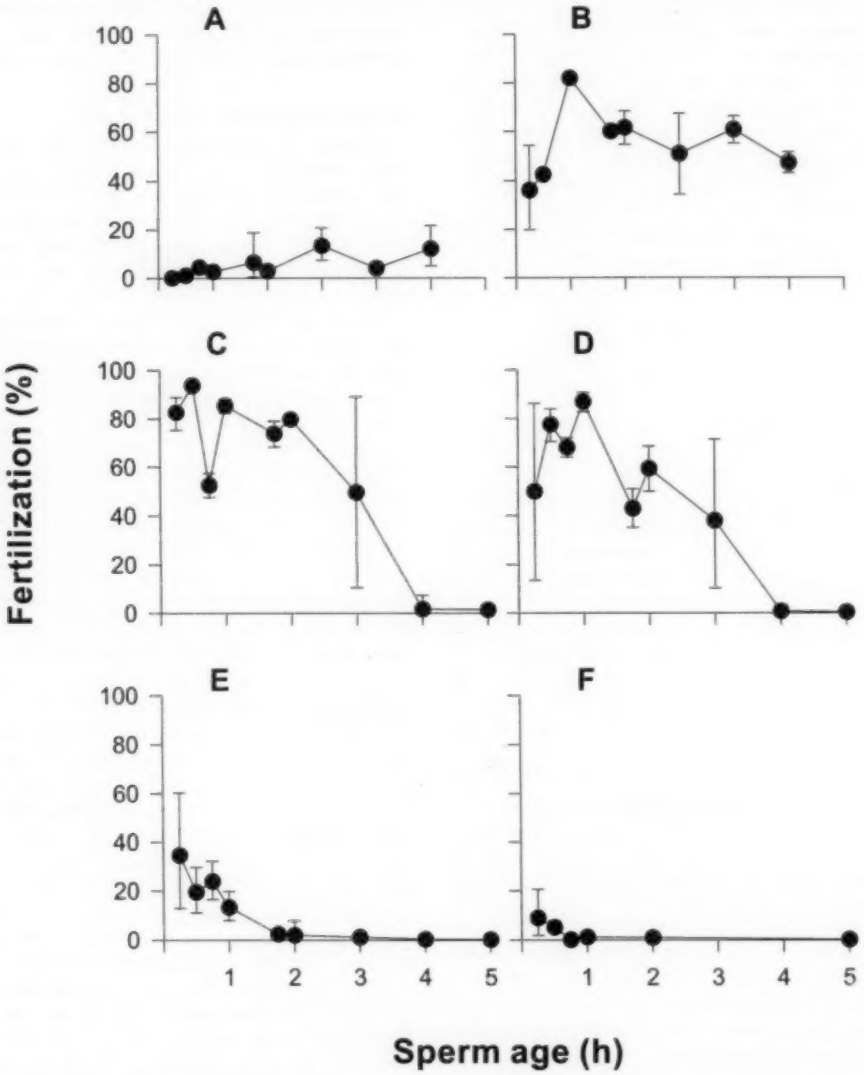
Fig. 5. Sperm age and sperm dilution for *H. laevigata*. Data are means ± 95% CI. (A) Dilution 0 = 1×10^7 sperm·mL^{-1}; (B) dilution 1 = 1×10^6 sperm·mL^{-1}; (C) dilution 2 = 1×10^5 sperm·mL^{-1}; (D) dilution 3 = 1×10^4 sperm·mL^{-1}; (E) dilution 4 = 1×10^3 sperm·mL^{-1}; (F) dilution 5 = 1×10^2 sperm·mL^{-1}.



$\alpha_y = 7.79$ and $\alpha_z = 0.77$ were used to estimate σ_y^2 and σ_z^2 by substitution (eqs. 4 and 5) following the method of Denny and Shibata (1989. These values were derived from measurements obtained in a separate study (Babcock et al. 2000) that examined dye diffusion rates at 5 m. For y,

$$(4) \qquad \sigma_y = \alpha_y \left(\frac{U^*}{\overline{U}} \right) x^{\beta}.$$

For z,

$$(5) \qquad \sigma_z = \alpha_z \left(\frac{U^*}{\overline{U}} \right) x^{\beta}.$$

Only the rate constant β (Denny and Shibata 1989) for the spread of the sperm plume was arbitrarily set at 0.5, as used by Babcock et al. (1994). None of the model parameters were varied a posteriori to optimize the fit to the field data (least-squares linear regression, $r^2 = 0.96$, $p = 0.00064$).

Spatial distribution

Haliotis laevigata at the study sites (Cathedral Rock and East Taylor's Island combined) were found at a mean density of 0.54 ± 2.1·m^{-2}. The average nearest-neighbor distance for these animals ($n = 106$) was 0.6 m, in contrast with an expected nearest-neighbor distance of 0.68 m if they had been distributed randomly (Table 2). The population was clearly aggregated, exhibiting a significant nearest neighbor

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Fig. 6. Sperm–egg contact time and sperm dilution for *H. laevigata*. Data are means ± 95% CI. Regression lines (dashed lines) are ±95% CI (dotted lines). (A) Dilution 0 = 1×10^7 sperm·mL^{-1}; (B) dilution 1 = 1×10^6 sperm·mL^{-1}; (C) dilution 2 = 1×10^5 sperm·mL^{-1}; (D) dilution 3 = 1×10^4 sperm·mL^{-1}; (E) dilution 4 = 1×10^3 sperm·mL^{-1}; (F) dilution 5 = 1×10^2 sperm·mL^{-1}. The slope is significantly different from 0 for dilutions 1 and 4.

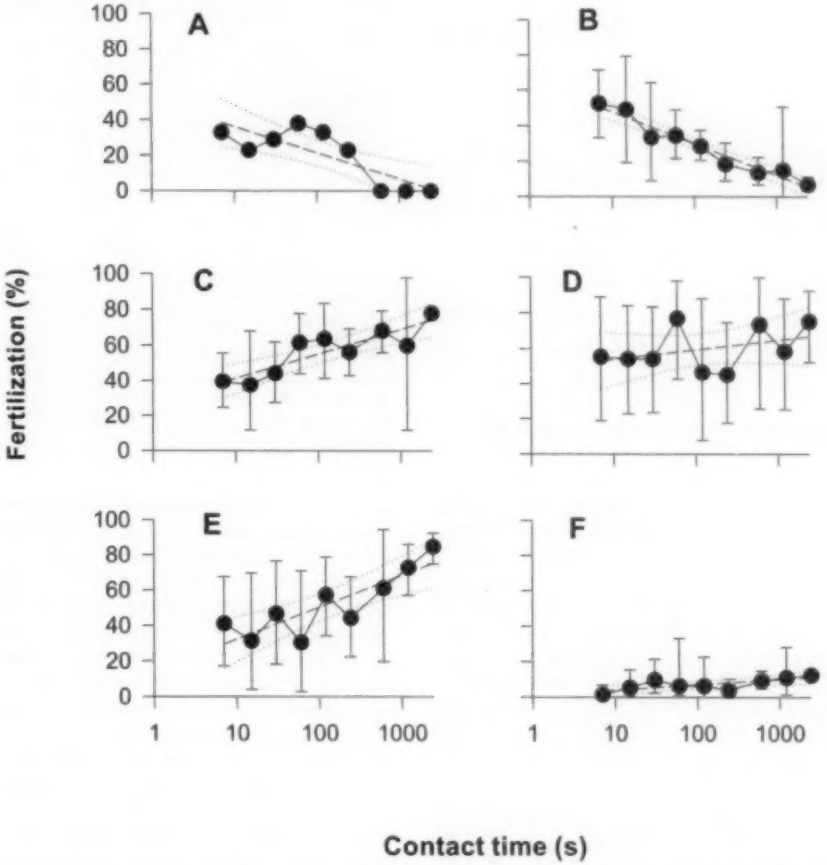
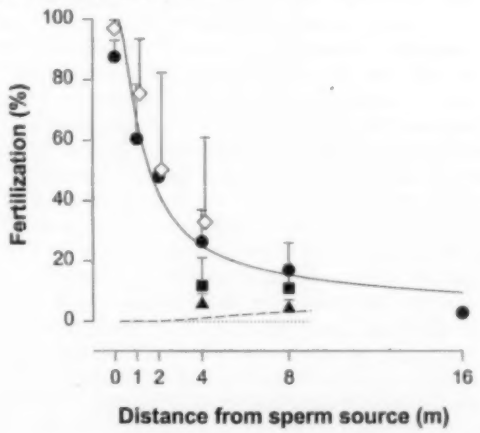


Fig. 7. Field fertilization rates for *H. laevigata*. Data are means ± 95% CI. Continuous current: ●, $y = 0$; ■, $y = 2$; ▲, $y = 4$. ◇, no net current. Model predictions: solid line, $y = 0$; dashed line, $y = 2$; dotted line, $y = 4$.



aggregation index R of 0.88 ($\chi^2 = -2.43$, $p < 0.05$; Clark and Evans 1954). A randomly distributed population would be expected to have an R value of 1.

Discussion

Haliotis laevigata is a relatively large abalone, releasing approximately an order of magnitude greater number of sperm than *Haliotis tuberculata* (Clavier 1992). This difference may be attributable to the difference in size of the species, since *H. tuberculata* is much smaller than *H. laevigata*; however, within *H. laevigata*, there was only a weak relationship between size and the number of gametes spawned, despite considerable shell length variation (Fig. 2). The number of eggs released per individual also appeared to be very similar, despite the large difference in the size of these species.

Peak fertilization rates, observed in vitro between 1×10^4 and 1×10^6 sperm·mL^{-1}, were in the same range as those observed in other abalone (Hahn 1989; Clavier 1992). Lower cell cleavage rates at higher concentrations are attrib-

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Fig. 8. Sperm half-life and sperm concentration for *H. laevigata*. Data are log-transformed half-lives and sperm concentrations from sperm age and sperm dilution experiments. Sperm half-life = sperm concentration × 0.19 ± 3.64, $r^2 = 0.82$.

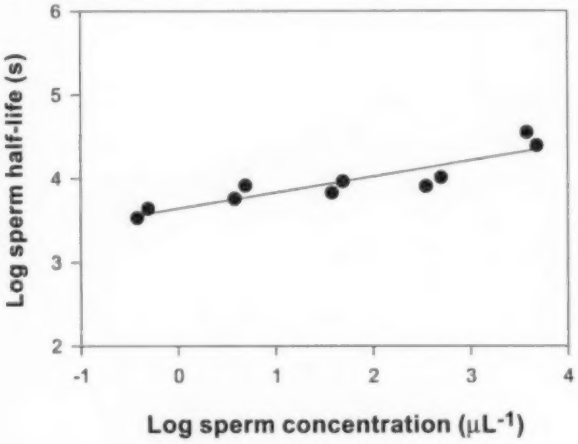

Table 2. Population densities and nearest-neighbor distances for *H. laevigata*.

Location	Density (m^{-2})	*R*	Nearest-neighbor distance (m)
Waterloo Bay			
100% synchrony	0.15	0.6	1.1
50% synchrony	0.15	0.6	1.6
West Island			
100% synchrony	0.3	0.8	1.0
50% synchrony	0.3	0.8	1.5
Cathedral Rock and Taylor's Island			
100% synchrony	0.5	0.88	0.9
50% synchrony	0.5	0.88	1.2

Note: Densities from Waterloo Bay (Shepherd and Partington 1995) and West Island (Shepherd and Brown 1993) are reported values at the time of stock collapse; those for Cathedral Rock and Taylor's Island are from stable commercially exploited populations at our study site. Values of *R* (nearest-neighbor aggregation index) are from Shepherd (1986) and from our data. Nearest-neighbor distances are calculated for male–female pairs (i.e., half the crude density) at either 50 or 100% population spawning synchrony.

uted to polyspermy, demonstrated in *Haliotis rufescens* by Stephano (1992), while almost no successful fertilizations were recorded below concentrations of 1×10^3 sperm·mL^{-1}. Gamete aging effects in *H. laevigata* were similar to those reported for other abalone, and eggs up to 2 h old appeared to show little effect of aging, as reported by Clavier (1992) for *H. tuberculata*. Sperm aging was much more rapid, particularly for dilute sperm suspensions, due to the respiratory dilution effect (Chia and Bickell 1983) in which sperm use up their available energy reserves and age more rapidly when in dilute suspensions. Sperm half-lives of >400 min were estimated for sperm concentrations of 1×10^6·mL^{-1}; however, both our empirical data and predictions of sperm diffusion models indicate that, in the field, sperm do not persist at these high densities for more than a few seconds, due to diffusion. Consequently, half-lives relevant to ecological considerations would appear to be at, or below, those reported here for dilute sperm suspensions, and sperm longevity is likely to be a minor component in determining fertilization rate relative to factors such as sperm dilution itself.

Sperm–egg contact time had little effect on fertilization rate, a surprising result in comparison with that found by Levitan et al. (1991) for *S. franciscanus* for which sperm–egg contact time was an important predictor of fertilization rate. Interestingly, even at the shortest contact times, fertilization rates were at levels of 30–50% (Fig. 5). Therefore, attachment of sperm to the egg may take place rapidly.

There have been numerous speculations on the implications of rapid sperm dilution for populations of abalone, but these have never been addressed in the field. Fertilization rates for *H. laevigata* measured in the field declined abruptly with increasing distance form the point of sperm release. These declines were not as abrupt as those for sea urchins (Pennington 1985; Levitan 1991) but were smaller than those for some starfish (Babcock et al. 1992, 2000). The relative rates of decline in fertilization success with distance observed in these groups are consistent with variation in sperm release rates. Sea urchins typically release sperm at rates in the range of 1×10^6·s^{-1} (Denny and Shibata 1989), abalone at 1×10^7·s^{-1} (Clavier 1992; this study), and large starfish at 1×10^8·s^{-1} (Babcock et al. 1994, 2000).

The predictions of the fertilization and sperm diffusion models provide a reasonably good fit with the field fertilization rates (Fig. 7), particularly for the $y = 0$ data (directly downstream). Given the empirical derivation of all but one of the parameters used in this model, this suggests that the model provides a robust means for estimating variations in fertilization success in natural populations. The fertilization model (eq. 1) is for a closed vessel, which, given the nature of our collecting apparatus, should best allow us to assess the predictive ability of the model. Our results must, however, be qualified by the consideration of several factors. In natural situations, there will be advection of both male and female gametes and continual diffusion and dilution. Our results could therefore be considered as conservative, since they may constrain gametes or predict higher fertilization rates than would actually be encountered between natural spawning pairs. Instantaneous fertilization rates (Denny and Shibata 1989, eq. 29) may be substantially lower than those that we have predicted and measured; however, it must be remembered that during natural spawnings, sperm will be available from several sources and this will have an additive effect on fertilization success (Levitan and Young 1995).

Abalone typically spawn in a series of as many as 70 contractions spaced over the spawning period (Clavier 1992) rather than in a continuous stream, as is the case in starfish and most sea urchins. Under natural spawning conditions the sperm release rate of abalone, and consequently the distribution of sperm in the field, is likely to be highly variable. This type of variability is not well accounted for by steady-state models, such as the one used here, that operate on average conditions. For the purposes of this paper, however, we assume that even though sperm concentrations, and conse-

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quently the fertilization rates experienced by individual female abalone, may vary considerably as a result of spawning behavior, these can be reduced to an overall population mean. Thus the model can form the basis for attempts to understand fertilization success at the population level (e.g., Levitan and Young 1995).

Both modeling and field results for *H. laevigata* from a range of hydrodynamic regimes indicate that rapid change in fertilization rate with distance takes place when individuals are separated by 1–4 m, with results from the hydrodynamic model also indicating a rapid reduction in fertilization rate between 1 and 4 m. In populations where spawning individuals are separated by distances of 1.6 m or greater, fertilization may be expected to be 50% or less and decrease rapidly with increasing distance (Fig. 7). These data may have considerable significance for the future management of *H. laevigata* stocks. Abalone populations at the study site have supported a fishery for many years and appear to be stable. Densities in this area were $0.54 \pm 2.12 \cdot m^{-2}$ during our study and nearest-neighbor distances averaged 0.6 ± 1.02 m. However, during spawning, the nearest individual of the opposite sex is likely to be even further away, since abalone are dioecious.

Clearly, the effective nearest-neighbor distance at the time of spawning is dependent on the level of spawning synchrony in the population. Because there are no published records of *H. laevigata* spawning behavior in the wild, we must rely on information from another species, *Haliotis kamtschatkana*, for an indication of intrapopulation spawning synchrony in wild abalone. Reports of spawning in this species indicate that entire local populations may participate in spawning (Stekoll and Shirley 1993), but other reports suggest that smaller proportions of the population may participate at other times ("more than half," Breen and Adkins 1980). It may therefore be reasonable to assume that the degree of spawning synchrony of *H. laevigata* varies between 100 and 50%. Assuming either 100% spawning synchrony or 50% synchrony, we have calculated the male–female nearest-neighbor distances for spawning individuals in our study population and for two other South Australian abalone populations at Waterloo Bay and West Island (Table 2).

Commercially viable populations at both Waterloo Bay and West Island have suffered recruitment collapse after being fished down to densities of $0.15 \cdot m^{-2}$ at Waterloo Bay (Shepherd and Partington 1995) and $0.3 \cdot m^{-2}$ at West Island (Shepherd and Brown 1993). At these densities, values for male–female nearest-neighbor distances were between 1 and 2 m in the Waterloo Bay and West Island spawning populations, while those for the study area at Cathedral Rock and Taylor's Island were 1 m or less (Table 2). Given the apparent role of aggregation in spawning induction in this species (Table 1), it is possible to speculate that reductions in density may further reduce reproductive success by limiting the ability to synchronize reproductive behavior. The congruence of critical densities for fertilization success (Fig. 7) and recruitment failure supports the suggestion (Shepherd and Partington 1995) that Allee effects have played a role in the collapse of the Waterloo Bay and West Island populations. Further advances in understanding the importance of such effects depend as much on an improved understanding of abalone spawning behaviors, such as population synchrony, as on refining our knowledge of gamete interactions.

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References

Allee, W.C. 1931. Animal aggregations: a study in general sociology. University of Chicago Press, Chicago, Ill.

Babcock, R.C., Mundy, C., Keesing, J., and Oliver, J. 1992. Predictable and unpredictable spawning events: in situ behavioral data from free-spawning coral reef invertebrates. Invertebr. Reprod. Dev. **22**: 213–228.

Babcock, R.C., Mundy, C.N., and Whitehead, D. 1994. Sperm diffusion models and in situ confirmation of long-distance fertilization in the free-spawning asteroid *Acanthaster planci*. Biol. Bull. (Woods Hole), **186**: 17–28.

Babcock, R., Franke, E., and Barr, N. 2000. Depth related variation in fertilization rates of the sea star *Coscinasterias calamaria*. Mar. Freshwater Res. In press.

Benzie, J.A.H., and Dixon, P. 1994. The effects of sperm concentration, sperm:egg ratio, and gamete age on fertilization success in crown-of-thorns starfish (*Acanthaster planci*). Biol. Bull. (Woods Hole), **186**: 139–152.

Brazeau, D.A., and Lasker, H.R. 1990. Sexual reproduction and external brooding by the Caribbean gorgonian *Briareum asbestinum*. Mar. Biol. **104**: 465–474.

Breen, P.A., and Adkins, B.E. 1980. Spawning in a British Columbia population of northern abalone *Haliotis kamtschatkana*. Veliger, **23**: 177–179.

Caddy, J.F. 1988. A perspective on the population dynamics and assessment of scallop fisheries, with special reference to the sea scallop *Placopecten magellanicus* Gmelin. *In* Marine invertebrate fisheries: their assessment and management. *Edited by* J.F. Caddy. John Wiley & Sons, New York. pp. 559–574.

Chia, F.S., and Bickell, L.R. 1983. Echinodermata. *In* Reproductive biology of invertebrates. Vol. II. Spermatogenesis and sperm function. *Edited by* K.G. Adiyodi and R.G. Adiyodi. John Wiley & Sons, Toronto, Ont. pp. 545–620.

Clark, P.J., and Evans, F.C. 1954. Distance to nearest neighbor as a measure of spatial relationships in populations. Ecology, **35**: 445–453.

Clavier, J. 1992. Fecundity and optimal sperm density for fertilization in the ormer (*Haliotis tuberculata* L). *In* Abalone of the world. *Edited by* S.A. Shepherd, M.J. Tegner, and S.A. Guzman del Proo. Blackwell, Oxford, U.K. pp. 86–94.

Denny, M.W., and Shibata, M.F. 1989. Consequences of surf-zone turbulence for settlement and external fertilization. Am. Nat. **134**: 859–889.

Ebert, T.A. 1998. An analysis of the importance of Allee effects in management of the red sea urchin *Strongylocentrotus franciscanus*. *In* Echinoderms: Proceedings of the Ninth International

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Echinoderm Conference, San Francisco, Calif. *Edited by* R. Mooi and M.Telford. Balkema, Rotterdam, The Netherlands. pp. 619–627.

Hahn, K.O. 1989. Handbook of culture of abalone and other marine gastropods. CRC Press, Boca Raton, Fla.

Levitan, D.R. 1991. Influence of body size and population density on fertilization success and reproductive output in a free-spawning invertebrate. Biol. Bull. (Woods Hole), **181**: 261–268.

Levitan, D.R. 1995. The ecology of fertilization in free-spawning invertebrates. *In* Ecology of marine invertebrate larvae. *Edited by* L. McEdward. CRC Press, New York.

Levitan, D.R., and Young, C.M. 1995. Reproductive success in large populations: empirical measures and theoretical predictions of fertilization in the sea biscuit *Clypeaster rosaceus*. J. Exp. Mar. Biol. Ecol. **190**: 221–241.

Levitan, D.R., Sewell, M.A., and Chia, F.S. 1991. Kinetics of fertilization in the sea urchin *Strongylocentrotus franciscanus*: interactions of gamete dilution, age, and contact time. Biol. Bull. (Woods Hole), **181**: 371–378.

Lillie, F.R. 1915. Studies on fertilization. VII. Analysis of variations in the fertilization power of sperm suspensions of *Arbacia*. Biol. Bull. (Woods Hole), **28**: 229–257.

Mundy, C.N., Babcock, R.C., Ashworth, I., and Small, J. 1994. A portable, discrete-sampling submersible plankton pump and its use in sampling starfish eggs. Biol. Bull. (Woods Hole), **186**: 168–171.

Munro, J.L. 1993. Giant clams. *In* Nearshore marine resources of the South Pacific. *Edited by* A. Wright and L. Hill. Forum Fisheries Agency, Honiara, Guadalcanal, Solomon Islands. pp. 431–449.

Pennington, J.T. 1985. The ecology of fertilization of echinoid eggs: the consequences of sperm dilution, adult aggregation, and synchronous spawning. Biol. Bull. (Woods Hole), **169**: 417–430.

Quinn, J.F., Wing, S.R., and. Botsford, L.W. 1993. Harvest refugia in marine invertebrate fisheries: models and applications to the red sea urchin, *Strongylocentrotus franciscanus*. Am. Zool. **33**: 537–550.

Rodda, K.R., Keesing, J.K., and Foureur, B.L. 1997. Variability in larval settlement of abalone on artificial collectors. Molluscan Res. **18**: 253–264

Sewell, M.A., and Levitan, D.R. 1992. Fertilization success during natural spawning of the dendrochirote sea cucumber *Cucumaria miniata*. Bull. Mar. Sci. **51**: 161–166.

Shepherd, S.A. 1986. Studies on southern Australian abalone (genus *Haliotis*). VII. Aggregative behavior of *H. laevigata* in relation to spawning. Mar. Biol. **90**: 231–236.

Shepherd, S.A., and Brown, L.D. 1993. What is an abalone stock: implications for the role of refugia in conservation. Can. J. Fish. Aquat. Sci. **50**: 2001–2009.

Shepherd, S.A., and Partington, D. 1995. Studies on southern Australian abalone (genus *Haliotis*). XVI. Recruitment, habitat and stock relations. Mar. Freshwater Res. **46**: 669–680.

Stekoll, M.S., and Shirley, T.C. 1993. In situ spawning behavior of an Alaskan population of pinto abalone *Haliotis kamtschatkana* Jonas, 1845. Veliger, **36**: 95–97.

Stephano, J.L. 1992. A study of polyspermy in abalone. *In* Abalone of the world. *Edited by* S.A. Shepherd, M.J. Tegner, and S.A. Guzman del Proo. Blackwell, Oxford, U.K. pp. 518–526.

Tong, L.J., and Moss, G.A. 1992. The New Zealand culture system for abalone. *In* Abalone of the world. *Edited by* S.A. Shepherd, M.J. Tegner, and S.A. Guzman del Proo. Blackwell, Oxford, U.K. pp. 583–591.

Vogel, H., Czihak, G., Chang, P., and Wolf, W. 1982. Fertilization kinetics in sea urchin eggs. Math. Biosci. **58**: 189–216.

Yund, P.O. 1990. An in situ measurement of sperm dispersal in a colonial marine hydroid. J. Exp. Zool. **253**: 102–106.

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Contribution of *Gammarus lacustris* to phosphorus recycling in a fishless alpine lake

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Abstract: We estimated the net P transport by *Gammarus lacustris* from the benthic to pelagic regions of a fishless alpine lake and compared it with P regeneration by the entire plankton community. *Gammarus lacustris* released between 5.2 and 18.1 ng $P{\cdot}L^{-1}{\cdot}h^{-1}$ (adults only and adults plus immatures, respectively) in the pelagic region during nighttime vertical migration. Additional P released into and removed from the water column due to predation on zooplankton was estimated at 1.87 and 2.3 ng $P{\cdot}L^{-1}{\cdot}h^{-1}$, respectively. The net daily regeneration of 52.2–181.4 ng $P{\cdot}L^{-1}{\cdot}day^{-1}$ by the *G. lacustris* population represented 9.5–32.9% of the total P regenerated by the planktonic community. The majority of the P released by *G. lacustris* represents "new" P to the pelagic zone because it originated in sediments. We conclude that *G. lacustris* can represent an important link in benthic–pelagic coupling in oligotrophic mountain lakes.

Résumé : Nous avons estimé le transport net de phosphore par *Gammarus lacustris* des régions benthiques aux régions pélagiques d'un lac alpin dépourvu de poisson, et nous l'avons comparé à la régénération du phosphore par l'ensemble de la communauté planctonique. *Gammarus lacustris* libérait entre 5,2 et 18,1 ng $P{\cdot}L^{-1}{\cdot}h^{-1}$ (adultes seulement, et adultes plus immatures, respectivement) dans la région pélagique pendant la migration verticale nocturne. La quantité supplémentaire de phosphore libérée et prélevée dans la colonne d'eau à cause de la prédation exercée sur le zooplancton a été estimée à 1,87 ng $P{\cdot}L^{-1}{\cdot}h^{-1}$ et 2,3 ng $P{\cdot}L^{-1}{\cdot}h^{-1}$ respectivement. La régénération quotidienne nette de 52,2 à 181,4 ng $P{\cdot}L^{-1}{\cdot}h^{-1}$ par la population de *G. lacustris* représentait 9,5 % à 32,9 % de la quantité de P total régénérée par la communauté planctonique. La plus grande partie du P libéré par *G. lacustris* représente du « nouveau » P pour la zone pélagique car il provient des sédiments. Nous en concluons que *G. lacustris* peut représenter un lien important du couplage benthique-pélagique dans les lacs de montagne oligotrophes.

[Traduit par la Rédaction]

Introduction

Phosphorus is typically the limiting nutrient for primary production in temperate freshwaters (Schindler 1978). Much effort has been expended to estimate the contribution of various parts of the biotic community to the cycling of P within lakes. For example, past attention focussed on the role of zooplankton as a major source of P in the epilimnion of lakes (e.g., Peters and Rigler 1973; Lehman 1984), although it is now known that protists and bacteria dominate nutrient regeneration in the plankton (Dodds et al. 1991; Hudson and Taylor 1996). Recently, bioenergetic modelling and fish manipulations have been used in an attempt to quantify the importance of fish and the effect of food web structure on P cycling (e.g., Schindler et al. 1993; Schaus et al. 1997). However, some researchers have suggested that regeneration of P by fish is insignificant (Nakashima and Leggett 1980; Mazumder et al. 1992). The release of P from sediments by benthivorous fish and its contribution to eutrophic conditions in shallow lakes are well known (e.g., Lougheed et al. 1998). The importance of P regenerated by fish has been assessed in relation to a variety of P sources such as that supplied by tributaries (Brabrand et al. 1990) but not in relation to the total amount of P regenerated (e.g., Hudson and Taylor 1996). We propose that the role of organisms in the cycling of P is put in context by comparing it with the planktonic P regeneration rate.

Benthic invertebrates also release P from sediments (Gallep 1979; Granéli 1979; Gardner et al. 1981). Of particular interest are species such as *Mysis* and *Chaoborus*, which migrate between the benthic and pelagic zones. Nutrients are removed from sediment and carried into the pelagic zone as the invertebrates migrate vertically. Phosphorus regenerated in this manner represents "new" P for pelagic consumers because it originated in the sediment. In fishless lakes of the Rocky Mountains, the amphipod *Gammarus lacustris* undergoes similar diurnal migrations (Anderson and Raasveldt 1974; Wilhelm and Schindler 1999). During the day, *G. lacustris* is epibenthic and feeds on sediment. At night, it migrates into the pelagic zone where it preys on zooplankton. Adult *G. lacustris* are large (15–18 mm long, 9–16 mg dry weight) and abundant (400 individuals$\cdot m^{-2}$), sometimes exceeding several thousand individuals per square metre (Anderson and Raasveldt 1974). Therefore, *G. lacustris* may represent an important benthic–pelagic link in oligotrophic alpine lakes by providing pelagic consumers with P recycled from sediments.

We estimated the net P regeneration of *G. lacustris* during its nocturnal migration into the pelagic zone in Snowflake

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Lake, an alpine lake in the Canadian Rocky Mountains. We measured the P released by *G. lacustris* experimentally. Phosphorus released into and removed from the water column due to predation on zooplankton was estimated from gut content analyses and published P values for zooplankton. We evaluated the net regeneration of P by *G. lacustris* by comparing it with the P regeneration rate of the plankton community. The planktonic regeneration rate was measured using the ^{33}P technique of Hudson and Taylor (1996). We conclude by comparing the contribution of the P regenerated by *G. lacustris* in Snowflake Lake with that of *Mysis relicta* in Lake Michigan.

Methods and materials

Study lake

Fishless Snowflake Lake is a small alpine lake (7.13 ha, mean depth 6.1 m, maximum depth 12.5 m, 2320 m above sea level) located in the eastern Front Ranges of the Canadian Rocky Mountains in Banff National Park (51°35′N, 115°50′W), Alberta, Canada. The lake is oligotrophic, with total P (TP) of 3–8 $\mu g \cdot L^{-1}$ and chlorophyll *a* of 0.1–2 $\mu g \cdot L^{-1}$. The waters are well buffered (pH values of 7.9–8.4, alkalinities of 900–1350 $\mu equiv. \cdot L^{-1}$). The lake stratifies weakly for short periods during the open-water season. High winds and snow during July and August can easily destratify the lake. Other chemical conditions and a map with detailed basin description can be found in McNaught et al. (1999).

The biotic community of Snowflake Lake has been manipulated several times through fish and zooplankton introductions. The lake was originally fishless but was stocked with salmonids for 5 years between 1960 and 1966. The presence of fish altered the zooplankton community from one dominated by *Hesperodiaptomus arcticus* and *Daphnia middendorffiana* to one dominated by *Diacyclops* and rotifers (Anderson 1972; McNaught et al. 1999). Fish did not reproduce in the lake and declined gradually, with the last fish caught in 1984. Once the lake was devoid of fish, *G. lacustris* and *D. middendorffiana* returned (McNaught et al. 1999), but the large copepod *H. arcticus* remained absent and small cyclopoid copepod species continued to dominate the zooplankton. *Hesperodiaptomus arcticus* was reintroduced on July 30, 1992, but did not become abundant in the plankton until late in 1996. *Hesperodiaptomus arcticus* reintroduction has caused the decline in abundance or elimination of all cyclopoid copepod and rotifer species (Wilhelm and Schindler 1999).

The benthic fauna is dominated by the amphipod *G. lacustris*, which is typically benthic by day and pelagic by night. While migrating, material ingested from benthic feeding on sediment is excreted and egested in the pelagic zone.

Gammarus lacustris density

Gammarus lacustris density in Snowflake Lake was determined by vertical net hauls of a Wisconsin-style plankton net (30 cm in diameter, 64-μm mesh size) and a bottom closing net (BCN) (0.168-m^2 opening, 500-μm mesh). Vertical hauls with the plankton net were taken on three dates in 1995 over the deepest point in the lake. On each date, three-stage hauls from each 0–3, 3–6, 6–8, and 8–11 m stratum were taken over a 24-h period at about 6-h intervals, starting between 12:00 and 14:00. Two additional whole-water column tows (0–11 m) were taken at each sampling time on two of those dates. In 1997, we took triplicate hauls at depths of 3, 7, and 11 m on five dates with the BCN during daylight hours only. We used the density of *G. lacustris* to estimate the regeneration rates of P by the amphipod population in the lake.

Movement of P from the benthic to the pelagic zone by *Gammarus lacustris*

Gammarus lacustris release of P derived from the sediment was measured experimentally in the field on July 17 and August 7, 1997. The July 17 run was a preliminary experiment to determine the amphipod density at which P release could be detected in a 2-h experiment. We collected *G. lacustris* with a dip net from a boat just after dusk, when individuals had migrated into surface waters with guts filled with sediment from daytime benthic feeding. Onshore, we rapidly transferred 10, 40, and 75 amphipods to duplicate polycarbonate Nalgene chambers containing 500 mL of lake water. All experimental chambers were filled with water from a common sample that was collected from the surface at an offshore location. Experimental chambers and syringes had previously been washed (0.1% Contrad-70), rinsed (ethanol), and leached (0.1 N HCl). A density of 75 amphipods per chamber was selected. This experimental density was greater than ambient densities in Snowflake Lake. For the August 7, 1997, experiment, we transferred 75 amphipods to each of eight experimental chambers. Four chambers were filled with adults only and another four chambers were filled with a mixture of adults and immatures. Two control chambers contained lake water only. All chambers were incubated partially submerged in a water bath filled with lake water. The water in the water bath was changed once during each experiment to avoid possible temperature fluctuations.

To determine P release rates, 35-mL water samples were removed with a plastic syringe before, immediately after (time zero), and at 5, 15, 45, and 120 min after introducing the amphipods. We were careful to only withdraw surface water to avoid fecal pellets released by the amphipods. The time and volume of water removed from each chamber were recorded. At the end of each experiment, the remaining water was decanted and the amphipods dried to a constant mass. TP in each water sample was analyzed using the method of Parsons et al. (1984). Our TP values do not include large particles that were generated by the amphipods, and we view the change in TP over time comparable with planktonic regeneration (see below). The change in TP in a chamber over the course of an incubation was used to calculate the net release rate from *G. lacustris*. The release of TP over time was modelled with a quadratic function and the release rate was determined by taking the derivative of this function at time zero. We used the rate at time zero because we expected TP release to decline over time, as we did not provide the amphipods with food. Results were combined into two groups: adults only and adults plus immatures.

Gammarus lacustris P regeneration from preying on zooplankton

While *G. lacustris* are in the water column at night, they prey on zooplankton (Wilhelm and Schindler 1999). Thus, predation on zooplankton and subsequent migration of *G. lacustris* to the sediment represents the removal of P from the pelagic zone. This would decrease the effect of new P released during the upward migration. We did not measure removal from the pelagic zone experimentally due to logistic constraints but estimated it as follows. From nighttime *G. lacustris* collections and gut content analyses in 1995, it was found that *Daphnia* made up 96% (by number) of all zooplankton consumed by *G. lacustris*. Over the 10-h period that *G. lacustris* spent in the water column, adults consumed an average of four 2-mm *D. middendorffiana* (F.M. Wilhelm, unpublished data). We used these data to estimate P dynamics as a result of zooplankton predation. *Daphnia middendorffiana* length was converted to dry weight using Edmondson's (1955) *D. middendorffiana* length–weight regression. *Daphnia* P content was estimated using Andersen and Hessen's (1991) value of 1.43% P of dry weight. Because *G. lacustris* does not ingest zooplankton whole, but rather in bits by shredding it, some food (and P) is lost before ingestion

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Table 1. Mean weight-specific P release rates of *G. lacustris* from Snowflake Lake at 10°C.

G. lacustris (no. of individuals)	Date in 1997	Dry weight (mg·individual^{-1}) Mean	SE	TP regeneration rate (ng P·mg^{-1}·h^{-1}) Mean	SE	*n*
Adults (10)	July 17	7.20	0.83	34.8	24.0	2
Adults (40)	July 17	7.70	0.59	22.8	1.9	2
Adults (75)	July 17	6.80	0.17	83.1	33.7	2
Adults (75)	August 7	6.23	0.03	45.6	13.0	4
Adults + immatures (75)	August 7	4.48	0.27	209.1	91.5	4

Note: Amphipods were collected at dusk after migrating to the lake surface; *n*, number of replicates.

Table 2. Phosphorus release rates of *G. lacustris*, other amphipods, other benthic invertebrates, and fish.

Species	P release rate (ng P·mg^{-1}·h^{-1})	Temperature (°C)	Source
Amphipods			
G. lacustris adults	45.6±13.0 SE (range 18.6–72.5)	10	This study
G. lacustris adults + immatures	209.1±91.5 SE (range 55.6–474.7)	10	
Pontoporeia hoyi	2.5–2.8	5	Nalepa et al. 1983
Lembos intermedius	1009.2, 0 h postfeeding	—	Johannes 1964
	489.6, 2 h postfeeding		
	161.8, 18 h postfeeding		
Other invertebrates			
Mysis relicta	29.0–409.0	4–7	Madeira et al. 1982
Chironomids[a]	29.7, full gut	20	Gardner et al. 1981
	27.6, cleared gut	20	
	13.0, full gut	5	
	8.4, cleared gut	5	
Chironomids[a]	16.4–18.6	16	Nalepa et al. 1983
Tubificids[a]	10.5, full gut	20	Gardner et al. 1981
	9.6, cleared gut	20	
	5.6, full gut	5	
	7.2, cleared gut	5	
	214.7 (0–30 min)[b]	5	
	56.0 (30–60 min)[b]	5	
Tubificids[a]	4.0–5.6	16	Nalepa et al. 1983
Fish			
Lepomis macrochirus	1.0–2.3, unfed	18–22	Mather et al. 1995
	1.2–5.7, fed	18–22	
Dorosoma cepedianum	1.0–1.3, unfed	19–21	Mather et al. 1995
	2.0–13.7, fed	19–21	

[a]Rate per milligram of ash-free dry weight, 24-h experimental duration.
[b]Time after removal from culture aquaria, short-term experiments.

(sloppy feeding). Although food loss due to sloppy feeding has not been examined for *G. lacustris*, we conservatively estimated this value at 40% based on loss rates for *Bythotrephes* and *M. relicta*, two raptorial zooplankton predators that also exhibit sloppy feeding (Lehman 1993; Smokorowski et al. 1998). Lehman (1993) reported that P ingestion efficiency by *Bythotrephes* was 58% (42% loss) when feeding on ^{33}P-labelled *Daphnia rosea*. Smokorowski et al. (1998) reported that mysids only ingested 31.7% of ^{113}Cd and 13.8% of ^{65}Cu when fed labelled *Daphnia magna*, indicating an approximate loss rate of 70–85%. This is considerably higher than the loss estimated by Lehman (1993) for P and may indicate differences between P and metals and (or) differences in the degree of sloppy feeding between the predators. Thus, we consider a loss rate of 40% as conservative.

The ingested material is then assimilated to some degree, while the remainder is egested and excreted. Amphipod assimilation rates vary with food type, ranging from 0 to 40% for leaves and up to 87% for algae and bacteria (Nilsson 1974; Lopez and Elmgren 1989). However, none of these include zooplankton food. Lehman (1993) argued that the selection of soft prey tissues (the apparent sloppy feeding) by *Bythotrephes* led to a highly assimilable diet (85% for C). Smokorowski et al. (1998) reported *Mysis* assimilation efficiencies of 72 and 21% for ^{113}Cd and ^{65}Cu, respectively. To be conservative, we used an assimilation efficiency of 85% for *G. lacustris*; a lower assimilation efficiency would increase P release to the water column as a result of zooplankton feeding. We assumed that half of the nonassimilated portion of dietary P was released in an available form via excretion to the water, while the other half was sequestered in a temporarily nonavailable form, e.g., fecal pellets.

Planktonic P regeneration in the water column

Detailed methods for measuring total planktonic P regeneration are outlined in Hudson and Taylor (1996). Briefly, on August 13,

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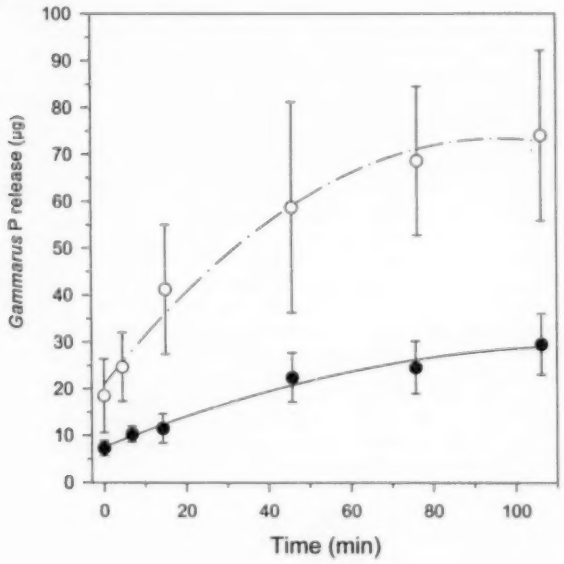
Table 3. Phosphorus regeneration of *G. lacustris* when feeding on sediment and zooplankton in Snowflake Lake in relation to planktonic TP regeneration.

	Amphipod density (individuals·L^{-1})	+TP regeneration from sediment	+TP regeneration from zooplankton predation	–Zooplankton TP assimilated	±Zooplankton TP excreta
Adults (75)	0.02 (0.003–0.052)	5.68 (0.85–14.77)	1.68 (0.25–4.51)	2.14 (0.32–5.56)	0.19 (0.03–0.49)
Adults + immatures (75)	0.02 (0.003–0.052)	18.6 (2.8–46.0)			
Planktonic rate[b]		23.0			

Note: The range in parentheses is based on the range of population density. All P release rates are in ng P·L^{-1}·h^{-1}; +, –, and ± indicate P addition or removal.

[a]Calculated assuming a constant planktonic rate throughout the day and a 10-h interval of *G. lacustris* migration into water column in August.
[b]Without *G. lacustris*.

Fig. 1. Sediment-derived P release by *G. lacustris* in Snowflake Lake. Seventy-five amphipods were incubated in situ in 500-mL chambers. A quadratic regression was fitted to P release. The derivative of the regression was then taken and the P release rate was determined at time zero. ●, adults only, μg P·min^{-1} = –1.47 × 10^{-3}·min^2 + 0.358·min + 7.49, r^2 = 0.98; ○, adults plus immatures, μg P·min^{-1} = –5.68 × 10^{-3}·min^2 + 1.09·min + 20.97, r^2 = 0.98. Means ± SE of four replicates for each treatment are presented.



1997, lake water (20 L) was removed from the surface waters from the middle of Snowflake Lake and placed into a clear polyethylene container that had been precleaned as described above. Water was then transported to the laboratory (~8 h by helicopter and car). In the laboratory, lake water (4 L) was placed into a clear precleaned polyethylene container. This water was incubated at ambient temperature (11°C) with carrier-free radiophosphate ($^{33}PO_4$, ~1000 Bq·mL^{-1}, ICN Biomedicals) for about 35 h to label the planktonic community, which did not include any *G. lacustris*. Incubations were terminated with the injection of a competitive inhibitor ($^{31}PO_4$, final concentration 1 mg P·L^{-1}). This prevented reincorporation of ^{33}P that was released from the plankton. The increase in ^{33}P in the dissolved pool over time was then measured. The slope of this line provided an estimate of the release rate of dissolved ^{33}P. The lake water remaining in the 20-L field container was analyzed for TP (Parsons et al. 1984). The release rate of dissolved ^{31}P from the planktonic community was calculated from the relationship

$$^{31}P \text{ released}/^{33}P \text{ released} = {}^{31}TP/{}^{33}TP$$

with the following formula:

$$^{31}P \text{ release rate} = {}^{33}P \text{ release rate} \times {}^{31}TP/{}^{33}TP$$

because at equilibrium:

$$^{31}TP/^{33}TP = {}^{31}TPP/{}^{33}TPP = {}^{31}TDP/{}^{33}TDP$$

where TPP is total particulate P and TDP is total dissolved P. Therefore, our definition for P regeneration was the transfer of P from the particulate pool (>0.2 μm) to the dissolved pool (<0.2 μm) over time. Egestion, excretion, exudation, decay, cell lysis, and sloppy feeding all contribute to this process. Activity was measured with a liquid scintillation counter and corrected for background counts. Quenching of samples was not present.

Net P regeneration by *G. lacustris*

We obtained the net P regeneration by *G. lacustris* from the pelagic zone as follows:

ng P·L^{-1}·day^{-1}
= ((ng P·mg^{-1}·h^{-1} × mg·individual^{-1} × individuals·L^{-1})
+ (ng P·L^{-1}·h^{-1} release from predation on zooplankton)
– (ng P·L^{-1}·h^{-1} assimilation + ng P·L^{-1}·h^{-1} fecal pellet production))
× 10 h·day^{-1}.

The first term accounts for the population regeneration of P from excretion and egestion of sediment derived from the benthic zone. The density term (individuals·L^{-1}) is the density of *G. lacustris* in the lake. The last two terms apportion the amount of P released into and exported from the pelagic zone due to predation on zooplankton. We then multiply by 10 h, the time spent per a 24-h period in the water column. Gut passage time was determined to be about 10 h, based on findings from 24-h surveys (F.M. Wilhelm, unpublished data).

Results

Gammarus lacustris density

In August 1995, the average nighttime *G. lacustris* density in the water column was 0.02 individual·L^{-1}, not including juveniles, of which only three were caught in all hauls. Juveniles rarely migrate in Snowflake Lake (F.M. Wilhelm,

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Total TP regenerated	Total TP lost from zooplankton predation	Net *G. lacustris* TP regenerated as a fraction of hourly planktonic regeneration (%)	Net *G. lacustris* TP regenerated as a fraction of daily planktonic regeneration (%)[a]
7.55 (1.13–19.77)	2.33 (0.35–6.05)	22.7 (3.4–59.7)	9.5 (1.4–24.9)
20.47 (3.08–51.0)	2.33 (0.35–6.05)	78.9 (11.9–195.4)	32.9 (4.9–81.4)

unpublished data). Densities estimated from the daytime BCN hauls in 1997 ranged from 0.003 to 0.052 individual·L^{-1} and encompass the density estimate obtained from nighttime vertical hauls in 1995. Because we did not perform nighttime hauls in 1997, we used the 1995 densities to estimate the regeneration of P by the amphipod population.

Gammarus lacustris P regeneration from sediment

The amount of P released by *G. lacustris* decreased over time (Fig. 1). Mean weight-specific P release rates for the July 17 preliminary experiment ranged from 34.8 to 83.1 ng P·mg dry weight^{-1}·h^{-1} (Table 1). On August 7, 1997, we measured mean weight-specific P release rates of 45.6 and 209.1 ng P·mg dry weight^{-1}·h^{-1} for adults only and adults plus immatures, respectively (Table 1). No mortality occurred during either experiment.

The mean dry weight of adult amphipods in the July 17 experiment ranged from 6.8 to 7.7 mg (Table 1). Mean dry weight of adults on August 7 was slightly lower at 6.2 mg, while the mean dry weight of individuals in the adult plus immature chambers was 4.5 mg (Table 1).

Phosphorus regeneration by *G. lacustris* preying on zooplankton

The mass of four 2.0-mm *D. middendorffiana* was estimated to be 146.6 µg, which resulted in an hourly ingestion rate of 14.66 µg *Daphnia*·*Gammarus*$^{-1}$·h^{-1}. This represented a P ingestion rate of 209.6 ng P·*Gammarus*$^{-1}$·h^{-1}, assuming that 1.43% of dry weight in *Daphnia* was P (Andersen and Hessen 1991). Of this, 93.2 ng P·*Gammarus*$^{-1}$·h^{-1} (40% loss due to sloppy feeding plus half of the unassimilated portion) was released into the water column, 106.9 ng P·*Gammarus*$^{-1}$·h^{-1} was assimilated, and 9.4 ng P·*Gammarus*$^{-1}$·h^{-1} was sequestered in fecal material.

Planktonic regeneration of P

The planktonic P regeneration rate (without amphipods) in Snowflake Lake was 23 ng P·L^{-1}·h^{-1} on August 13, 1997. A second rate measured on July 23, 1998, but not included in our calculations, was 28.2 ng P·L^{-1}·h^{-1}.

Net P regeneration by *G. lacustris*

The net P regeneration rate by the *G. lacustris* population in Snowflake Lake was 52.2 ng P·L^{-1}·day^{-1} (range 7.8–137.2) and 181.4 ng P·L^{-1}·day^{-1} (range 27.3–449.5) for adults only and adults plus immatures, respectively. This represented between 9.5 and 32.9% of the daily planktonic regeneration rate, assuming a constant planktonic regeneration rate over 24 h.

Discussion

Our measured release rates of P for *G. lacustris* fell within the range of P release rates reported for other invertebrates (Table 2). However, direct comparisons between our measured P release rates and other studies are difficult because P release rates are highly variable and often reflect methodological and environmental differences. *Gammarus lacustris* release rates of P were up to 15 times higher than P release rates of fed fish (Table 2). Our planktonic regeneration rate is similar to the mean regeneration rates of P (36.0 and 40.0 ng P·L^{-1}·h^{-1}) reported for two small oligotrophic lakes (TP = 3.2–13 µg·L^{-1}) in southcentral Ontario (Hudson and Taylor 1996). Our rate is also similar to a planktonic regeneration rate of 28.9 ng P·L^{-1}·h^{-1} reported for Flathead Lake, a large oligotrophic (TP ≈ 5.5 µg·L^{-1}) lake in northwestern Montana (Dodds et al. 1991). The slightly higher rates of P regeneration in the other lakes compared with our alpine lake may be due to warmer water temperatures or methodological differences.

On a daily basis, P regeneration by *G. lacustris* was 9.5–32.9% (range 1.4–81.4%) of the planktonic regeneration rate (Table 3). This indicates that *G. lacustris* can be an important source of new P to the pelagic zone in Snowflake Lake. The areal density of *G. lacustris* in Snowflake Lake is intermediate for alpine lakes at 400 individuals·m^{-2} (Parker et al. 1996). Densities of several thousand per square metre occur in some lakes (Anderson and Raasveldt 1974). At such high densities the amount of P transported and released in the epilimnion could exceed planktonic regeneration rates.

Most of the P released by *G. lacustris* in the pelagic zone originated in bottom sediments, so that it represents the addition of new P to the pelagic zone. Caraco et al. (1992) estimated that about 35% of the summer algal biomass in Mirror Lake (New Hampshire) required a source of new P, in addition to external sources of P and that recycled within the pelagic zone. The authors measured sources of P that are rarely measured, such as P input from groundwater and bathers, in an attempt to balance their P budget. They hypothesized that vertically migrating invertebrates such as *Chaoborus* may be an important source of P but did not quantify it. Our results suggest that migrating benthic invertebrates are key factors in benthic–pelagic interactions and provide a mechanism by which nutrients from the benthos

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are made available to pelagic consumers. Our findings underscore the need for aquatic ecologists to expand pelagic food web studies to include benthic–pelagic linkages.

Several possible errors are involved in our method of measuring P regeneration by *G. lacustris*. These include the removal of organisms from food, the evacuation of gut contents over time, and crowding (Peters and Rigler 1973; Mullin et al. 1975; Lehman 1984). The removal from food and evacuation of gut contents are problematic in studies with small-bodied species of zooplankton because of their high metabolic rates and short gut-clearance times. As a result, nutrient release rates quickly decline (Korstad 1983). Our estimate of P release is taken at the beginning of the incubation (time zero) to minimize this bias. We collected the amphipods and started the experiments shortly after they had migrated to the surface, when their guts were still full. *Gammarus lacustris* gut evacuation over the 2-h experimental period was not complete, so our rates should closely approximate ambient release rates.

Crowding of organisms can either increase or decrease the estimated release rates (Peters and Rigler 1973; Mullin et al. 1975). Peters and Rigler (1973) and Korstad (1983) observed decreased release rates at high zooplankton density and attributed this to physiological stress from overcrowding. However, Mullin et al. (1975) attributed large differences in nutrient excretion by crowded compared with individual zooplankters to nutrient leaching due to crowding. We did not directly address artifacts resulting from overcrowding in this study. Our results indicate a slight, but weak, positive relationship between P release and *Gammarus* density (Table 1). The different rates between the two treatments on August 7, 1997, may also stem from crowding. However, the higher release rate in the adult plus immature treatment chambers could be due to a higher weight-specific excretion rate of the smaller immatures. A more likely explanation is the release of P by injured organisms. *Gammarus lacustris* adults usually prey on the smallest conspecifics (young-of-the-year), but aggression towards immatures has also been observed (F.M. Wilhelm, personal observation). Therefore, antagonistic interactions between adults and immatures may have resulted in the increased release of P.

Alternatively, body P may have been released by immatures when they were accidentally drawn into the syringe and injured as a sample was being withdrawn. When this occurred, the water and amphipod were returned to the chamber and a new sample was withdrawn. Although no mortalities occurred during the experiment, nonlethal injuries may have resulted in the release of body P. However, replicates where individuals were drawn into the syringe did not consistently demonstrate higher release rates than replicates where individuals were not drawn into the syringe. Thus, we think that the antagonistic activity among amphipods was the more important release process.

Due to these interactions, release rates from chambers containing immatures may be overestimates. However, P release from injured animals may also occur under natural conditions in Snowflake Lake. Dense amphipod aggregations that include all sizes of individuals occur in Snowflake Lake. Many individuals from these aggregations have damaged or missing limbs, an indication of aggressive behaviour. Therefore, we view our measured release rate with immatures as a maximum release rate. We consider our adult-only release rate as more typical of lake conditions.

Sloppy feeding by raptorial invertebrate predators results in a large proportion of uningested food and P. Nutrients in this uningested food may not be immediately available, e.g., that associated with the carapace, in which case, we have overestimated the sloppy feeding loss. However, Smokorowski et al. (1998) found that 32% of the ^{113}Cd in labelled *Daphnia* was released to soluble form after *M. relicta* had fed on them for 4 h. *Gammarus lacustris* feeding on *Daphnia* releases finely shredded material (F.M. Wilhelm, personal observation). Therefore, much of the P in the uningested food may be released. In addition, the small particles would have a slow sinking rate and thus be exposed to further breakdown and P release during their transition through the water column, especially since the mixed water column is quite deep. Even if all P from predation on *Daphnia* was exported to the benthos, *G. lacustris* would still represent a net source of P (1.48–14.4 ng $P \cdot L^{-1} \cdot day^{-1}$) to the water column (Table 3).

Comparison of P regeneration by vertically migrating invertebrates

The paucity of published P release rates for migrating invertebrates and a lack of adequate planktonic regeneration rates preclude a rigorous comparison of P regeneration by *G. lacustris* with that of vertical migrants in other lakes. However, Hudson et al. (1999) estimated planktonic regeneration rates when lake water TP is known from

$$\log_{10} \text{planktonic regeneration rate (ng } P \cdot L^{-1} \cdot h^{-1}) = 1.0077 \times \log_{10} \text{TP } (\mu g \cdot L^{-1}) + 0.7206.$$

We used this relationship to calculate the planktonic regeneration rate for Lake Michigan to compare the contribution of P recycled by *M. relicta*, for which release rates are available (Madeira et al. 1982). We calculated the TP in the epilimnion of Lake Michigan in 1977, when Madeira et al. (1982) measured *Mysis* release rates, by interpolating Rousar's (1973) 1970–1971 average of 8.9 $\mu g \cdot L^{-1}$ for station 2 and Shafer and Armstrong's (1994) July 1990 average of 5 $\mu g \cdot L^{-1}$ for the epilimnion to arrive at a TP value of 7.5 $\mu g \cdot L^{-1}$. This decline in TP between these studies is consistent with that reported for Lake Michigan between 1976 and 1984 as a result of P management (Scavia et al. 1986). Using the above equation, we estimated the Lake Michigan planktonic regeneration rate at 960.8 ng $P \cdot L^{-1} \cdot day^{-1}$. We calculated *M. relicta* release rates from Madeira et al. (1982, table 5) in nanograms of P per litre per day (their rates are corrected for time spent in the water column). Phosphorus regeneration rates by *M. relicta* ranged from 9.9 to 105.6 ng $P \cdot L^{-1} \cdot day^{-1}$. Thus, P regeneration by *M. relicta* accounted for 1.0–10.9% of the planktonic regeneration rate, lower than our rates for *G. lacustris*. Therefore, we conclude that P regeneration by *G. lacustris* in Snowflake Lake is more important than that of *M. relicta* in Lake Michigan. These differences may arise from a number of factors including population density, sediment P content, tissue stoichiometry, water temperature, and differences in methodology. It should also be noted that the rate calculated above for *M. relicta*

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does not account for the removal of P from predation on zooplankton. However, P removal from *M. relicta* predation on zooplankton should be similar to that for *G. lacustris*, given their analogous predation behaviour.

By using the planktonic regeneration rate as a basis for comparison, we were able to determine that *G. lacustris* is an important benthic–pelagic link in Snowflake Lake. Through its vertical migrations, it provides pelagic consumers with P recycled from lake sediments. The importance of other organisms in the regeneration of P in lakes could be evaluated similarly by comparing their P regeneration rates with the planktonic regeneration rate.

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References

Andersen, T., and Hessen, D.O. 1991. Carbon, nitrogen, and phosphorus content of freshwater zooplankton. Limnol. Oceanogr. **36**: 807–814.

Anderson, R.S. 1972. Zooplankton composition and change in an alpine lake. Verh. Int. Ver. Limnol. **18**: 264–268.

Anderson, R.S., and Raasveldt, L.G. 1974. *Gammarus* predation and the possible effects of *Gammarus* and *Chaoborus* feeding on the zooplankton composition in some small lakes and ponds in western Canada. Can. Wildl. Serv. Occas. Pap. No. 18.

Brabrand, A., Faafeng, B.A., and Nilssen, J.P.M. 1990. Relative importance of phosphorus supply to phytoplankton production: fish excretion versus external loading. Can. J. Fish. Aquat. Sci. **47**: 364–372.

Caraco, N.F., Cole, J.J., and Likens, G.E. 1992. New and recycled primary production in an oligotrophic lake: insights for summer phosphorus dynamics. Limnol. Oceanogr. **37**: 590–602.

Dodds, W.K., Priscu, J.C., and Ellis, B.K. 1991. Seasonal uptake and regeneration of inorganic nitrogen and phosphorus in a large oligotrophic lake: size-fractionation and antibiotic treatment. J. Plankton Res. **13**: 1339–1358.

Edmondson, W.T. 1955. The seasonal life history of *Daphnia* in an arctic lake. Ecology, **36**: 439–455.

Gallep, G.W. 1979. Chironomid influence on phosphorus release in sediment–water microcosms. Ecology, **60**: 547–556.

Gardner, W.S., Nalepa, T.F., Quigley, M.A., and Malczyk, J.M. 1981. Release of phosphorus by certain benthic invertebrates. Can. J. Fish. Aquat. Sci. **38**: 978–981.

Granéli, W. 1979. The influence of chironomid plumosus larvae on the exchange of dissolved substances between sediment and water. Hydrobiologia, **66**: 149–159.

Hudson, J.J., and Taylor, W.D. 1996. Measuring regeneration of dissolved phosphorus in planktonic communities. Limnol. Oceanogr. **41**: 1560–1565.

Hudson, J.J., Taylor, W.D., and Schindler, D.W. 1999. Planktonic nutrient regeneration and cycling efficiency in temperate lakes. Nature (Lond.), **400**: 659–661.

Johannes, R.E. 1964. Uptake and release of phosphorus by a benthic marine amphipod. Limnol. Oceanogr. **9**: 235–242.

Korstad, J. 1983. Nutrient regeneration by zooplankton in southern Lake Huron. J. Gt. Lakes Res. **9**: 374–388.

Lehman, J.T. 1984. Grazing, nutrient release, and their impacts on the structure of phytoplankton communities. *In* Trophic interactions within aquatic ecosystems. *Edited by* D.G. Meyer and J.R. Strickler. Am. Assoc. Adv. Sci. Publ. **85**: 49–72.

Lehman, J.T. 1993. Efficiencies of ingestion and assimilation by an invertebrates predator using C and P dual isotope labelling. Limnol. Oceanogr. **38**: 1550–1554.

Lopez, G., and Elmgren, R. 1989. Feeding depths and organic absorption for the deposit-feeding benthic amphipods *Pontoporeia affinis* and *Pontoporeia femorata*. Limnol. Oceanogr. **34**: 982–991.

Lougheed, V.L., Crosbie, B., and Chow-Fraser, P. 1998. Predictions on the effect of common carp (*Cyprinus carpio*) exclusion on water quality, zooplankton, and submergent macrophytes in a Great Lakes wetland. Can. J. Fish. Aquat. Sci. **55**: 1189–1197.

Madeira, P.T., Brooks, A.S., and Seale, D.B. 1982. Excretion of total phosphorus, dissolved reactive phosphorus, ammonia, and urea by Lake Michigan *Mysis relicta*. Hydrobiologia, **93**: 145–154.

Mather, M.E., Vanni, M.J., Wissing, T.E., Davis, S.A., and Schaus, M.H. 1995. Regeneration of nitrogen and phosphorus by bluegill and gizzard shad: effect of feeding history. Can. J. Fish. Aquat. Sci. **52**: 2327–2338.

Mazumder, A., Taylor, W.D., Lean, D.R.S., and McQueen, D.J. 1992. Partitioning and fluxes of phosphorus: mechanisms regulating the size-distribution and biomass of plankton. Arch. Hydrobiol. Bei. Ergeb. Limnol. **35**: 121–143.

McNaught, S.A., Schindler, D.W., Parker, B.R., Paul, A.J., Anderson, R.S., Donald, D.B., and Agbeti, M. 1999. Restoration of the food web of an alpine lake following fish stocking. Limnol. Oceanogr. **44**: 127–136.

Mullin, M.M., Perry, M.J., Renger, E.H., and Evans, P.M. 1975. Nutrient regeneration by some oceanic zooplankton: a comparison of methods. Mar. Sci. Commun. **1**: 1–13.

Nakashima, B.S., and Leggett, W.C. 1980. The role of fishes in the regulation of phosphorus availability in lakes. Can. J. Fish. Aquat. Sci. **37**: 1540–1549.

Nalepa, T.F., Gardner, W.S., and Malczyk, J.M. 1983. Phosphorus release by three kinds of benthic invertebrates: effects of substrate and water medium. Can. J. Fish. Aquat. Sci. **40**: 810–813.

Nilsson, L.M. 1974. Energy budget of a laboratory population of *Gammarus pulex* (Amphipoda). Oikos, **25**: 35–42.

Parker, B.R., Wilhelm, F.M., and Schindler, D.W. 1996. Recovery of *Hesperodiaptomus* from diapausing eggs following elimination by stocked salmonids. Can. J. Zool. **74**: 1292–1297.

Parsons, T.R., Maita, Y., and Lalli, C.M. 1984. A manual of chemical and biological methods for seawater analysis. Pergamon Press, Tarrytown, N.Y.

Peters, R.H., and Rigler, F.H. 1973. Phosphorus release by *Daphnia*. Limnol. Oceanogr. **18**: 821–839.

Rousar, D.C. 1973. Seasonal and spatial changes in primary production and nutrients in Lake Michigan. Water Air Soil Pollut. **2**: 497–514.

Scavia, D., Fahnenstiel, G.L., Evans, M.S., Jude, D.J., and Lehman, J.T. 1986. Influence of salmonine predation and weather on long-term water quality trends in Lake Michigan. Can. J. Fish. Aquat. Sci. **43**: 435–443.

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Schaus, M.H., Vanni, M.J., Wissing, T.E., Bremigan, M.T., Garvey, J.E., and Stein, R.A. 1997. Nitrogen and phosphorus excretion by detritivorous gizzard shad in a reservoir ecosystem. Limnol. Oceanogr. **42**: 1386–1397.

Schindler, D.E., Kitchell, J.F., He, X., Carpenter, S.R., Hodgson, J.R., and Cottingham, K.L. 1993. Food web structure and phosphorus cycling in lakes. Trans. Am. Fish. Soc. **122**: 756–772.

Schindler, D.W. 1978. Factors regulating phytoplankton production and standing crop in the world's freshwaters. Limnol. Oceanogr. **23**: 478–486.

Shafer, M.M., and Armstrong, D.E. 1994. Mass fluxes and recycling of phosphorus in Lake Michigan: role of major particle phases in regulating the annual cycle. *In* Environmental chemistry of lakes and reservoirs. *Edited by* L.A. Baker. American Chemical Society, Washington, D.C. pp. 285–322.

Smokorowski, K.E., Lasenby, D.C., and Evans, R.D. 1998. Quantifying the uptake and release of cadmium and copper by the opossum shrimp *Mysis relicta* preying upon the cladoceran *Daphnia magna* using stable isotope tracers. Can. J. Fish. Aquat. Sci. **55**: 909–916.

Wilhelm, F.M., and Schindler, D.W. 1999. Effects of *Gammarus lacustris* (Crustacea: Amphipoda) on plankton community structure in an alpine lake. Can. J. Fish. Aquat. Sci. **56**: 1401–1408.

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Intraspecific gastropod shell strength variation among north temperate lakes

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Abstract: Defensive morphological traits may vary intraspecifically. Freshwater snail shells are conspicuous defensive structures. In north-central Wisconsin, we investigate whether among-lake differences in shell strength relate to water chemistry or predator abundance and whether shell strength is inducible owing to predation risk from crayfish. *Amnicola limosa* shells were stronger in lakes with abundant crayfish predators. An experiment and a general understanding of prosobranch evolution suggest that this may result from selection rather than induction. The experiment indicated a weakening of shells of slow-growing *A. limosa* in the presence of crayfish. This may have resulted from resource depression caused by a strong behavioral response that reduced feeding time. *Physa skinneri* shell strength was correlated with lake calcium concentrations, and a weak trend with calcium was apparent for *Helisoma anceps*. Decreased *P. skinneri* shell strength in low-calcium lakes may result from retention of scarce calcium by the body at the expense of allocation to the shell. Populations of *H. anceps* differed in rates of shell strength increase with body size, suggesting that rates of shell strength accumulation with ontogeny vary among populations. Shell strength increased more rapidly in lakes with abundant predators than in lakes with few predators.

Résumé : Les caractères morphologiques défensifs peuvent varier à l'intérieur d'une même espèce. Les coquilles des gastropodes dulcicoles sont des structures défensives bien évidentes. Dans le centre-nord du Wisconsin, nous avons voulu établir si les différences dans la résistance des coquilles de divers lacs sont liées à la chimie de l'eau ou à l'abondance des prédateurs, et si le risque de prédation par les écrevisses pouvait accroître la résistance des coquilles. Les coquilles d'*Amnicola limosa* étaient plus résistantes dans les lacs abritant de nombreuses écrevisses prédatrices. Une expérience que nous avons réalisée et notre connaissance de l'évolution des prosobranches laissent penser que ce phénomène pourrait être dû à la sélection naturelle plutôt qu'à un processus d'induction. Notre expérience a montré qu'il y avait affaiblissement des coquilles d'*A. limosa* à croissance lente en présence d'écrevisses. Cela pourrait avoir été attribuable à une réduction importante du temps consacré à l'alimentation, et donc à l'absorption des substances assurant la résistance des coquilles, liée au comportement d'évitement des prédateurs. La résistance des coquilles de *Physa skinneri* était corrélée avec les concentrations lacustres de calcium, alors qu'on a observé à ce chapitre qu'une faible tendance dans le cas de *Helisoma anceps*. Il se pourrait que la résistance moindre des coquilles de *P. skinneri* dans les lacs pauvres en calcium soit due au fait que le peu de calcium absorbé par l'animal se trouve surtout retenu dans son corps au lieu d'être dirigé vers la coquille. Les taux d'accroissement de la résistance de la coquille avec la taille corporelle variaient d'une population à l'autre, ce qui laisse penser que le taux d'accroissement de la résistance au cours de l'ontogenèse varie d'une population à l'autre. La résistance des coquilles s'accroissait plus rapidement dans les lacs abritant de nombreux prédateurs.

[Traduit par la Rédaction]

Introduction

Animals have evolved a variety of defensive morphological traits that confound the handling and ingestion efforts of predators (e.g., Krueger and Dodson1981; Osenberg and Mittelbach 1989). Differences between conspecific populations may exist in how effectively morphological defenses deter predators (Johansson and Samuelsson 1994). These interpopulation differences may result from longer term selection for antipredator traits in some populations or from shorter term morphological plasticity. Regardless, environmental factors driving differences in morphology can modify predator–prey interactions.

Gastropods have served as model taxa for investigating intraspecific variation in morphology. These studies have revealed a pervasive dimorphism in which snail shell form differs owing to environmental variation in wave exposure and predation risk (Kitching and Lockwood 1974). The shell of freshwater snails is a conspicuous morphological defense that impedes shell-damaging predators. Species characterized by strong shells are less susceptible to being crushed by fish than weaker-shelled species are (Osenberg and Mittelbach 1989), and crayfish predation on many snail species requires breaking the shell to access edible tissue (Covich 1977). No studies, however, document intraspecific, interpopulation variation in freshwater gastropod shell strength or the possible causes of this variation.

Environmental factors that may influence the strength of freshwater snail shells include calcium and predators. First,

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Table 1. Environmental attributes of lakes from which snails were collected for shell strength analyses.

Lake	Calcium concentration ($mg \cdot L^{-1}$)	Pumpkinseed abundance ($no. \cdot trap^{-1} \cdot day^{-1}$)	Crayfish abundance ($no. \cdot trap^{-1} \cdot day^{-1}$)	Species crushed
Allequash (al)	12.21	0.93	8.07	1, 2
Big (bi)	18.08	0.34	26.66	1, 2
Big Muskellunge (bm)	6.05	0.00	14.54	1
Dads (da)	2.32	0.00	0.00	1
High (hi)	14.17	5.12	1.17	1
Laura (la)	7.41	0.00	2.95	1, 2, 3
Little Rice (lr)	5.60	na	na	3
Sparkling (sp)	10.16	0.00	5.98	2, 3
Tower (to)	4.27	28.37	0.00	2
Trout (tr)	12.40	0.16	27.40	1
West Plum (wp)	16.03	na	na	3
Wildcat (wi)	23.82	2.97	0.75	1, 2

Note: These variables were predictors in models of shell strength. Snail species crushed: 1, *A. limosa*; 2, *H. anceps*; and 3, *P. skinneri*. Lake name abbreviations are indicated in parentheses. na, data not available.

calcium strengthens snail shells by binding with carbonate, forming a lattice crystalline structure embedded in the shell's proteinaceous periostracum (Wilbur 1976). A long-standing hypothesis is that populations from calcium-rich waters will have shells with a greater calcium content, and thus stronger shells (Russell-Hunter et al. 1981). The physiological mechanism underlying this pattern is that snails will maintain a particular body tissue calcium concentration regardless of calcium availability in the water. The shell serves as a buffer against calcium loss to the relatively dilute water, resulting in thin shells in systems with extremely low ion concentrations (McMahon 1983). A positive correlation between environmental and shell calcium is observed in some pulmonate species but is only one of four possible relationships (Russell-Hunter et al. 1981). Shell response to calcium concentration in the medium is complex, varies among species, and remains a source of debate (Lodge et al. 1987).

Second, predators might alter the effectiveness of the shell as protection. This can result from an induced defensive response by prey organisms in the short term or selection favoring the persistence of predator-resistant lineages in the long term. Defensive morphology arising from induction and selection is common in a wide range of taxa and ecosystems (Havel 1987; Dodson 1989*a*; Baumgartner 1995). Some marine snails, owing to either induction (Appleton and Palmer 1988) or natural selection (Palmer 1979), have thicker shells when sympatric with abundant predators, while conspecifics from environments with fewer predators have thinner shells. Vulnerability to predation is less for thicker-shelled individuals (e.g., Kitching and Lockwood 1974).

A broad gradient in the chemical and predator characteristics that may influence freshwater snail shell strength is present throughout the Northern Highland Lake District in Wisconsin, U.S.A. Our purposes were to determine whether shell strength of snails differs among populations, whether any such differences are related to differences in water chemistry or predator abundance, and whether shell strength is an inducible trait owing to predation risk from crayfish.

Materials and methods

With about 2500 lakes, the northern Wisconsin portion of the Northern Highland Lake District is characterized by much among-lake variation in physical, chemical, and biological characteristics. Some of this variation is driven by the lakes' spatial setting in the regional hydrologic flow regime (Kratz et al. 1997). The relatively softwater lakes in this region range from <1 to 23 mg calcium$\cdot L^{-1}$. Crayfish (genus *Orconectes*) and pumpkinseed (*Lepomis gibbosus*), two important snail predators, range from absent to abundant (Capelli and Magnuson 1983; Rahel 1984). We selected lakes that would likely span gradients of crayfish (Capelli and Magnuson 1983), pumpkinseed (Mittelbach et al. 1992), and calcium concentration (inferred from Black et al. 1963). We then measured these variables directly (Table 1).

Measurement of calcium and predators

Calcium concentration and predators were measured once per lake during 1995–1996. Calcium was measured from 1 m depth in nearshore water and analyzed with atomic absorption spectrophotometry (Kenoyer and Bowser 1992). Calcium is a relatively conservative element through time (except during periods of extreme drought; Webster et al. 1996), demonstrating little temporal variation within a lake relative to variation among lakes (J.J. Magnuson, Long-Term Ecological Research Project, unpublished data).

Relative crayfish abundance was estimated using wire minnow traps with 40-mm-diameter openings and baited with 120 g of beef liver (Capelli and Magnuson 1983). In each lake, we set three to six clusters of five traps each, with traps 5 m apart. Traps were set at 1–2 m depths for 22–26 h. Clusters were deployed in three habitat types per lake, usually a submerged macrophyte site, a macrophyte-free cobble site, and a macrophyte-free sand site. Some lakes did not have all habitat types; these were usually lakes with few crayfish. Relative abundance was calculated as the number of *Orconectes* (*O. propinquus*, *O. rusticus*, and *O. virilis*) per trap per 24 h. We estimated relative pumpkinseed abundance using 7-mm-mesh fyke nets, 12 m long and with a first hoop opening of 0.9 × 0.75 m and an 8 × 1.25 m leader net. We set three to six nets per lake in the littoral zone (first hoop in water about 1.25 m deep) near the crayfish traps for 22–26 h. Pumpkinseed over 70 mm long are molluscivorous (Mittelbach 1984), so we calculated abundance as the number of fish greater than 70 mm long per net per 24 h. The interannual variability of these predator populations is generally not statistically significant during the period 1991–1998 in the North Temperate Lakes – Long-Term Ecological Research Project lakes (ANOVA of pumpkinseed or crayfish versus year for five lakes, p ranges from 0.12 to 0.88 except for two cases of non-directional fluctuations; J.J. Magnuson, unpublished data). Additionally, catch rates of pumpkinseed using fyke nets are significantly correlated with catch rates using electroshocking

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equipment ($r = 0.33$) and beach seines ($r = 0.29$, $n = 106$ year–lake combinations, $r_{0.05} = 0.27$) (J.J. Magnuson, unpublished data).

Evaluation of shell strength

We measured shell strength in the prosobranch *Amnicola limosa* and the pulmonates *Helisoma anceps* and *Physa skinneri* (Table 1), species that are ubiquitous among and abundant within Northern Highland lakes (D.B. Lewis, unpublished data). In July and August 1995, we collected *A. limosa* from the littoral zone of eight lakes, *H. anceps* from six lakes, and *P. skinneri* from four lakes. To determine whether shell strength differed among lakes, we first accounted for the effect of body size by measuring *A. limosa* and *P. skinneri* shell length and *H. anceps* shell diameter (hereafter referred to as length). Using live, damp snails maintained in their water of origin, we measured compressive shell strength as the force (newtons) necessary to crush the shell across the body whorl with a mass-loading device, modified from Osenberg and Mittelbach (1989). Pressure was exerted through the body whorl along an axis perpendicular to the columnella for *A. limosa* and *P. skinneri* and was exerted across the axis defined by the peripheral keels of the body whorl for *H. anceps*. For subsequent analyses, shell strength and shell length were $\log_e$ transformed.

Our objective was to investigate whether shell strength for each species varied among lakes and was correlated with environmental variables. It was necessary to initially account for the effect of body size on shell strength, but we could not assume that shell strength would scale with body size the same way in all lakes. Therefore, we used multiple regression models with interaction terms between body size and environmental effects. We modeled shell strength as a function of shell length, calcium concentration, crayfish abundance, pumpkinseed abundance, and the interactive effect of each environmental term with shell length. (For *P. skinneri*, length and calcium were the only main effects because predator abundances were not estimated in two of the lakes; see Table 1.)

Significance was determined using a partial F test, also known as an extra sum of squares test, to compare models in a hierarchically nested set (Draper and Smith 1981). With this approach, a full model including all main and interactive effects was fit. The amount of variance explained by any one effect was determined by the magnitude of increase in the model's residual sum of squares when that effect was excluded. For example, the variation explained by the "$\log_e$(length) × pumpkinseed" effect was determined by comparing the residual sum of squares from the full model with the residual sum of squares from the model with this term removed. Similarly, the variation explained by the "calcium" effect was determined by comparing the residual sum of squares from the model with all four main effects versus the model with calcium removed (higher order effects are not included when determining the significance of lower order effects; Wilkinson et al. 1992). The two residual sums of squares were compared with an F ratio (Draper and Smith 1981). This approach is called the type II sum of squares multiple regression (Wilkinson et al. 1992). There are no complications with the order of entry of terms into the model because the influence of each effect is determined separately (i.e., the focal effect is removed and all other effects of equal or lesser order are retained). The direct measure of an effect's influence on shell strength is the magnitude by which the model's residual sum of squares increases when the effect is removed. The F ratio adjusts this additional error relative to the degrees of freedom and is used to determine significance. For these reasons, we used the amount of error attributable to and the F ratio for each effect to rank environmental terms by their influence on shell strength. Direction of the effect of each term (+ or –) on shell strength was noted.

Shell strength induction experiment

We tested whether predation risk induced stronger *A. limosa* shells by comparing the shell crushing resistance of snails raised in two laboratory environments. One hundred and twenty (120) snails from an initially weak-shelled population (Allequash Lake) were cultured in water containing potential chemical cues produced by *O. rusticus* consuming *A. limosa*. An additional 120 were raised in aged control water lacking a cue. Snails were raised for 48 days from a mean initial damp mass including shell of 2.8 mg (SD = 0.9).

Snails were raised in 200-mL glass jars positioned randomly in incubators at 21.5°C on a 16 h light : 8 h dark cycle. Forty (40) incubation jars were used for each treatment, and each jar contained three snails. The food resource was periphyton, which grew readily on the bottom and sides of the jars. Incubation jars were inoculated daily with their respective treatment. The source water from Trout Lake, Wisconsin, was aerated in the laboratory for at least 7 days and then transferred to either 38-L aquaria lacking macroscopic animals (control water) or aquaria containing crayfish (predation risk treatment). The predation cue was prepared each day by feeding snails to crayfish. Immediately after crayfish ceased feeding, water was taken from the control and crayfish cue-generating aquaria and used to replace one third of the water in the appropriate incubation jars. After 48 days, shell length (millimetres), width (millimetres), damp mass (milligrams), and crushing resistance (newtons) were measured in all experimental snails. Mean growth for each jar was calculated as the mean initial mass of all snails in a jar subtracted from the jar mean final mass. Snail growth was not tracked individually because this would have required tagging or marking, which might have affected shell growth or introduced foreign chemicals to the jars. Individuals from the initial experimental population were analyzed, as snails did not reproduce.

Treatments were compared with linear regressions of $\log_e$ transformations of shell strength, width, and mass versus $\log_e$(shell length). In the interpretation of these analyses, a significant treatment effect would indicate that the shell strength versus length regression had different y-intercepts between control and crayfish-exposed snails. We were measuring only adult snails, however, with no individuals near $\log_e$(length) = 0. To avoid the related statistical problem of large differences in the y-intercept, we centered the data (sensu Bates and Watts 1988) by subtracting the mean length from each length value. In this analysis, if the regression lines for the two treatments crossed within the range of the data, it would suggest that snails reaching a smaller final size responded differently than those that grew to a larger size. If lines crossed for any of the three shell characteristics (strength, width, and mass), we grouped the jars into growth rate classes and repeated the multiple regression analysis for each growth rate class separately. For this analysis, shell strength and length data were jar means rather than values for individual snails. Data were untransformed and centered around the mean length of each growth class.

Results

Shell strength

Snail species and body size were important determinants of freshwater snail shell strength, but substantial variability remained unexplained (Fig. 1). For *A. limosa*, shell strength and the rate at which it increased with body size varied among lakes (Fig. 2*a*; Table 2). In the multiple regression model, crayfish and calcium were significant as main effects (Table 3). Crayfish abundance was strongly positively correlated with shell strength, and calcium concentration showed a weaker, negative correlation with shell strength. The variability in shell strength explained by crayfish abundance was over six times greater than the amount explained by calcium concentration (Table 3). No interactive effects were significant (Table 3), indicating that the environmental variables

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Fig. 1. Shell strength as a function of shell length. Data points represent the shell length and strength of individual snails. Regression lines and parameters represent each species aggregated from all lakes. For *A. limosa*, intercept = 0.108 and slope = 1.735, for *H. anceps*, intercept = –0.801 and slope = 1.382, and for *P. skinneri*, intercept = –3.539 and slope = 2.128.

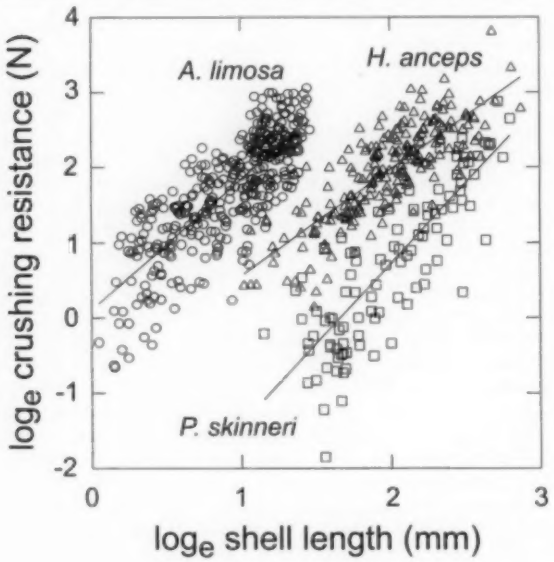
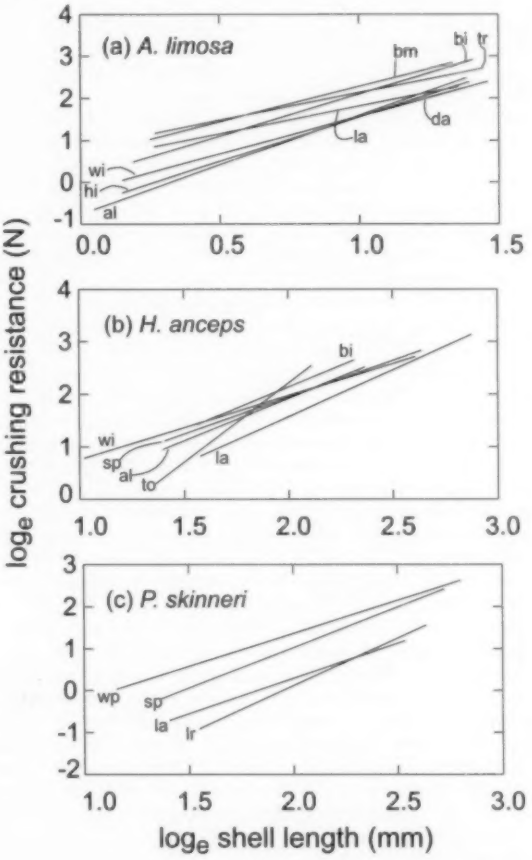


Fig. 2. Regression lines of crushing resistance versus shell strength for (*a*) *A. limosa*, (*b*) *H. anceps*, and (*c*) *P. skinneri*. For each species, each line represents a different lake. Lines are labeled with lake name abbreviations from Table 1. Tests of homogeneity of slopes: (*a*) $p < 0.001$, (*b*) $p < 0.001$, and (*c*) $p = 0.185$.



that we measured did not explain variation in the slopes of shell strength versus length (Fig. 2*a*).

Helisoma anceps shell strength and its rate of increase with body size also varied among lakes (Fig. 2*b*; Table 2). In the regression model, all environmental main effects were significant, although none explained a great deal of variability (Table 4). Calcium concentration was positively related to shell strength and explained slightly more variation than other environmental terms. Interactive terms of both calcium and pumpkinseed abundance with shell length were significant (Table 4).

Physa skinneri shell strength varied among lakes, yet the rate at which strength increased with body size was similar (Fig. 2*c*; Table 2). Calcium was very strongly positively related to shell strength, but its interactive effect with body size was not significant (Table 5).

The three species differed by virtue of how strongly shell strength was correlated with an environmental parameter. The positive relationship between *P. skinneri* shell strength and calcium concentration was the strongest. Also strong, but less so, was the positive relationship between *A. limosa* shell strength and crayfish abundance. The environmental parameters that we measured explained little variability among lakes in *H. anceps* shell strength.

Induction experiment

Relative to control snails, snails exposed to crayfish cues grew less (D.B. Lewis, unpublished manuscript). Comparing shell strength and allometry between treatments, however, required comparably sized snails. Therefore, we limited our analysis to snails with shell lengths in the range of overlap between the two treatments (2.5–4.0 mm; $n = 82$ control snails, $n = 103$ crayfish-exposed snails). There was no treatment or treatment × length effect on shell width or damp snail mass. Both the treatment ($p = 0.047$) and treatment × length ($p = 0.002$) terms had significant effects on shell crushing resistance, indicating that the intercepts and slopes of the shell strength versus length regression differed between treatments (Fig. 3). The lines for the two treatments crossed within the range of the data (Fig. 3), suggesting that snails reaching a smaller final size responded differently than those reaching a larger size. Growth rates were roughly bimodal (Fig. 4), so we grouped jars into two growth rate classes: < 7 mg gain over the 48-day experiment and ≥ 7 mg gain. Within each growth rate class, jar mean shell strength was regressed against jar mean length, treatment, and the length × treatment interaction. Analysis again was limited to jars with a mean length inside the range of overlap between treatments (2.6–3.8 mm). Length had no significant effect on shell strength for either growth rate class (Table 6). For the slow-growth group, snails reared in the crayfish cue environ-

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Table 2. Number of snails crushed, the strength and length ranges of the snails, parameters for crushing resistance regression, and regression R^2 for each species and lake.

Species	Lake	No. of snails	Force range (N)	Length range (mm)	β_0 (SE)	β_1 (SE)	R^2
A. limosa	al	48	0.5–14.3	1.0–4.0	–0.76 (0.13)	2.35 (0.13)	0.87
	bi	49	0.7–19.7	1.2–4.1	0.10 (0.14)	2.00 (0.14)	0.80
	bm	43	2.4–20.0	1.3–3.8	0.61 (0.08)	1.67 (0.09)	0.89
	da	12	5.0–11.8	2.9–4.1	–0.27 (0.86)	1.83 (0.70)	0.41
	hi	36	0.5–14.4	1.2–4.0	–0.57 (0.17)	2.12 (0.17)	0.81
	la	54	1.6–13.3	1.3–3.9	0.51 (0.10)	1.31 (0.11)	0.72
	tr	50	2.9–21.3	1.3–4.3	0.83 (0.11)	1.31 (0.10)	0.77
	wi	56	0.9–13.7	1.2–4.3	–0.23 (0.15)	1.79 (0.15)	0.72
H. anceps	al	49	1.5–23.8	4.0–10.7	–1.31 (0.30)	1.62 (0.15)	0.72
	bi	48	2.6–20.8	4.8–10.2	–1.11 (0.63)	1.62 (0.32)	0.36
	la	23	1.7–27.6	4.8–17.8	–1.99 (0.53)	1.78 (0.22)	0.75
	sp	22	2.4–16.7	4.1–14.0	–0.85 (0.38)	1.40 (0.18)	0.76
	to	29	1.1–12.2	4.0–8.3	–3.81 (0.56)	3.01 (0.31)	0.78
	wi	34	1.5–20.5	2.8–13.6	–0.46 (0.23)	1.22 (0.13)	0.74
P. skinneri	la	24	0.3–4.4	4.1–12.6	–3.10 (0.65)	1.69 (0.38)	0.47
	lr	24	0.2–4.8	4.7–14.0	–4.45 (0.58)	2.28 (0.28)	0.75
	sp	41	0.7–17.7	3.9–15.3	–2.86 (0.31)	1.94 (0.14)	0.83
	wp	16	0.8–14.2	3.2–16.5	–1.80 (0.50)	1.58 (0.25)	0.74

Note: The regression model was $\log_e(\text{force}) = \beta_0 + \beta_1 \cdot \log_e(\text{length})$; $p < 0.001$ in all cases except for *A. limosa* from Dad's Lake ($p = 0.025$). See Table 1 for lake abbreviations.

Table 3. Results of a multiple regression testing the interactive effects of shell length and environmental variables on *A. limosa* shell strength.

Effect tested	Direction of effect	RSS	Additional error	Partial F ratio	p
None (full model)		45.4			
$\log_e$(length) × pumpkinseed	+	45.8	0.4	2.8	ns
$\log_e$(length) × crayfish	Variable	45.4	0.0	0.2	ns
$\log_e$(length) × calcium	+	45.7	0.3	2.6	ns
None (main effects)		47.5			
Pumpkinseed	–	47.9	0.4	3.2	ns
Crayfish	+	55.1	7.6	54.7	<0.001
Calcium	–	48.7	1.2	8.8	<0.005
$\log_e$(length)	+	179.4	131.8	952.5	<0.001

Note: The effect tested indicates which term was excluded, and the residual sum of squares (RSS) for that model is indicated. The magnitude of increase in the model's RSS is denoted as the additional error attributable to the exclusion of the effect. F ratios and significance of interactive effects are calculated from comparison of RSS between the restricted and full models. In determining significance of main effects, the "full" model includes all four main effects but no interactive terms. Thus, several models were fit with slightly different combinations of predictor variables. We report the direction of each term's effect on shell strength as being positive (+), negative (–), or variable among models. ns, not significant.

ment had weaker shells than snails in the control (Fig. 5*a*). Treatments did not differ significantly in the fast-growth group (Fig. 5*b*). There was no length × treatment interaction (i.e., slopes did not differ between treatments) for either growth rate category (Table 6).

Discussion

For the freshwater snail species examined, an antipredator morphological attribute varied among lakes. This variation was linked to environmental factors, but the important factor differed among snail species. Crayfish abundance is the environmental variable most highly correlated with *A. limosa* shell strength in these lakes, but increased shell strength was not induced in individuals exposed to predation risk cues. *Physa skinneri* shell strength correlated with lake calcium concentration, and *H. anceps* shell strength may have also correlated to a slight degree. For *A. limosa* and *H. anceps*, not only does shell strength vary among populations, the scaling of shell strength with body size varies as well. This suggests that populations differ with respect to the rate of strength accumulation with ontogeny. If differences among

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Table 4. Results of multiple regression model of *H. anceps* shell crushing resistance (see note to Table 3 for details).

Effect tested	Direction of effect	RSS	Additional error	Partial *F* ratio	*p*
None (full model)		23.6			
$\log_e$(length) × pumpkinseed	+	25.4	1.8	14.9	<0.001
$\log_e$(length) × crayfish	+	23.8	0.2	1.9	ns
$\log_e$(length) × calcium	–	24.3	0.7	5.8	<0.05
None (main effects)		26.6			
Pumpkinseed	+	27.4	0.8	5.9	<0.05
Crayfish	+	27.7	1.1	8.0	<0.005
Calcium	+	28.0	1.4	10.2	<0.005
$\log_e$(length)	+	75.1	48.5	363.7	<0.001

Table 5. Results of multiple regression model of *P. skinneri* shell crushing resistance (see note to Table 3 for details).

Effect tested	Direction of effect	RSS	Additional error	Partial *F* ratio	*p*
None (full model)		17.5			
$\log_e$(length) × calcium	+	18.1	0.6	3.5	ns
Calcium	+	36.9	18.8	105.7	<0.001
$\log_e$(length)	–	84.2	66.1	372.4	<0.001

Note: In this analysis, the model testing $\log_e$(length) × calcium (i.e., the model with this term excluded) is equivalent to the main effects only model.

Fig. 3. Shell strength versus shell length relationship for snails raised in water containing a crayfish predation cue and in control water lacking a cue.

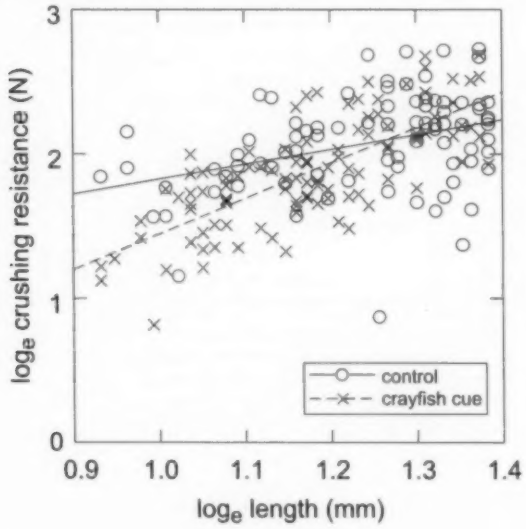
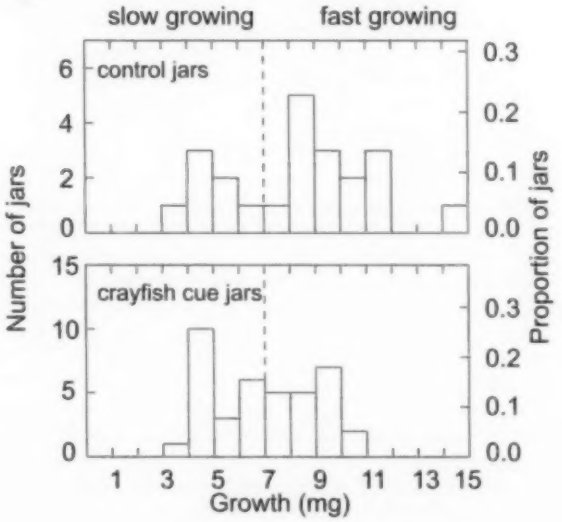


Fig. 4. Growth of control and crayfish-exposed snails over a 48-day duration. Growth increments are depicted in 1-mg ranges. Bars denote the number of jars in which snails exhibited a given growth increment. The broken vertical line at growth = 7 mg indicates the dividing point between slow- and fast-growing snails.



populations in shell strength reflect differences in vulnerability to ingestion by predators, these environmental variables may modify snail–predator interactions.

Response of *A. limosa*

Predation risk from crayfish may influence *A. limosa* shell strength. Predators cause the development of defensive morphological characteristics in prey by at least three mechanisms. First, a predation-resistant morphology can be the result of natural selection. When predators are abundant, they act as a strong selective force for prey with effective defenses (Vermeij et al. 1981). Second, predation risk can directly elicit an induced morphological response in prey. That is, individuals can directly alter their own morphology in

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Table 6. Sample size (number of jars) and p values from regression of jar mean shell strength versus jar mean shell length, treatment, and length × treatment for both slow and fast growth rate categories.

Statistic	Slow-growth group	Fast-growth group
Control n	7	15
Crayfish cue n	20	19
Length p	0.301	0.546
Treatment p	0.047	0.371
Length × treatment p	0.932	0.360

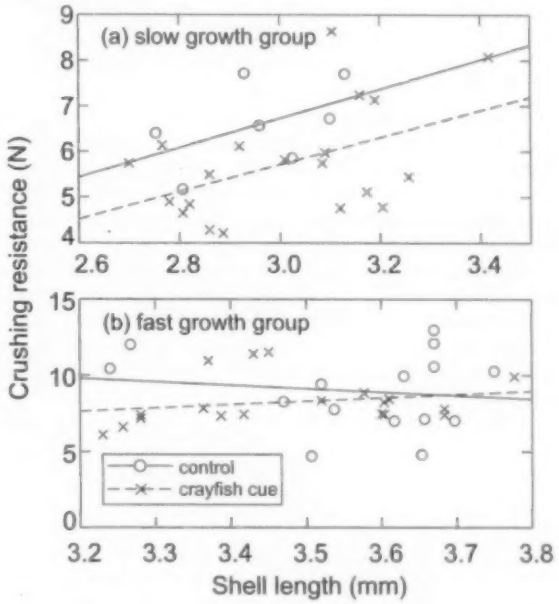
response to the presence of predators (Appleton and Palmer 1988; Dodson 1989*a*). A third possible mechanism is embryological induction, in which an organism displays predator-resistant morphological attributes owing to the environment in which its parents lived (Krueger and Dodson 1981; Dodson 1989*b*).

Although predation risk can induce defensive behavioral and life historical responses in freshwater gastropods (Crowl and Covich 1990; Covich et al. 1994; Turner et al. 1999), our experiment suggests that *O. rusticus* does not induce increased shell strength in *A. limosa*. This conclusion is based on at least one important assumption. We assumed that the Trout Lake water in the predator-free control bore no predation risk chemical cues. Snails in the predator cue treatment showed a pronounced behavioral response and growth depression relative to the control snails (D.B. Lewis, unpublished manuscript). Crayfish-exposed snails exhibited a vertical migration, crawling up the sides of the glass jar and often upside down on the surface tension of the water. This behavior was very rarely observed in control snails, indicating that there was likely no cue present in the aerated control water.

A comparison between snails reared in crayfish cue versus control water led to some counterintuitive results. For slow-growing snails, shell strength was weaker in individuals exposed to odors of their predators. This effect was dampened with the faster-growing snails. Two important questions emerge. First, why did some prey organisms, contrary to our expectation, respond with weakened morphological defenses when confronted with predation risk (Fig. 5*a*)? Second, why was this effect dampened in fast-growing prey (Fig. 5*b*)?

These results appear consistent with the observation that growth and behavior respond to both predation risk and growth potential (Werner et al. 1983). Among the jars receiving the crayfish cue, there was variability in the frequency and extent of the crawl-up behavioral response. Growth rates were lower in snails exhibiting a stronger behavioral response, indicating that feeding rates were reduced in animals that responded strongly (D.B. Lewis, unpublished manuscript). Slow-growing *A. limosa* in the crayfish cue treatment would have experienced the greatest predation risk. These individuals were small and in an environment with predators (as indicated by chemical cues). As a result, they demonstrated a very strong behavioral response, so likely ate less and incorporated less energy. One consequence of less energy intake would be weaker shells, relative to control snails, for a given body size (Fig. 5*a*).

Fig. 5. Crushing resistance versus shell length relationship for (*a*) slow-growing and (*b*) fast-growing snails. Each point represents the mean shell strength and mean length of snails within one jar.



Resource depression resulting from a strong behavioral response may thus explain why morphological defenses were weakened in the presence of predators. This result is contrary to morphological induction, in which prey improve defensive morphological traits in response to predation risk. This finding supports the hypothesis that the positive relationship between crayfish abundance and *A. limosa* shell strength observed among lakes results from natural selection.

Shell strength depression in fast-growing *A. limosa* was not as severe as in slow-growing snails (Fig. 5*b*). Fast-growing snails in the crayfish cue treatment were larger, so they would experience less predation risk relative to their slower-growing counterparts. The faster-growing snails demonstrated a less pronounced behavioral response (D.B. Lewis, unpublished manuscript). Thus, they were likely able to feed more. A greater incorporation of energy would have allowed them to allocate resources to both body tissue and shell growth. As a consequence, the shell strength of fast-growing treatment snails approached that of the control snails. Why were some jars characterized by fast-growing snails that exhibited a only a mild behavioral response, while others contained slower-growing snails that responded more strongly? The answer may lie in initial conditions. Although there was no statistical difference between slow-growing and fast-growing snails in shell length, width, or mass at the start of the experiment, mass and width (and, by extension, shell strength) were slightly greater in the group that subsequently grew more rapidly. Fast-growing snails started 4.1% heavier and 2.3% wider than slow-growing snails did. Collectively, these traits may have initially conferred just enough apparent protection for some snails to re-

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spond less strongly, spend more time foraging, and start on a faster growth trajectory. These subtle initial differences in behavior and predation risk (i.e., size and shell strength) may have increased over the duration of the experiment, resulting in final differences of a much greater magnitude.

Snails in the control group experienced a predation risk of zero. A greater amount of energy was available to control snails, allowing them to allocate to both tissue and shell growth, thus producing shells of greater strength for a given size. On a mortality risk – growth potential gradient (sensu Werner et al. 1983), slow-growing snails exposed to predation risk lie at one extreme, snails not exposed to predators lie at the other, and fast-growing individuals exposed to predation risk are intermediate.

Consistent with the hypothesis that molluscivory drives the evolution of morphological defenses in freshwater snails (Vermeij and Covich 1978), we adopt natural selection as the favored explanation for the among-lake correlation between *A. limosa* shell strength and crayfish abundance. A competing explanation is induction, but the laboratory experiment suggests that this does not occur. In fact, the opposite may occur for resource-limited individuals. Generalizations of freshwater gastropod evolution further support our hypothesis of selection for shell strength by predators. As a prosobranch, *A. limosa* likely evolved in large, permanent water bodies such as lakes (McMahon 1983). Across Northern Highland lakes, crayfish abundance is variable owing to several environmental gradients (Capelli and Magnuson 1983; Kershner and Lodge 1995). Thus, among *A. limosa* populations, there has probably been a gradient of interaction strength with crayfish spanning their evolutionary history, potentially generating among-population differences in shell strength. Genetic control over shell morphology is evident in other snail species as well. Reciprocal transfer of individuals between different field environments did not eliminate intraspecific, interpopulation variation in ratios of aperture length or width to shell length in other species (Nickerson 1972; Hunter 1975).

Response of pulmonates

The relationship between snail shell calcium content and environmental calcium concentration may take at least four forms (Russell-Hunter et al. 1981). To the extent that shell calcification conveys crushing resistance, the shell strength – environmental calcium relationship may reflect the same four patterns. One is the direct positive correlation observed here with *P. skinneri*. In this case, internal tissue calcium content is stable across media of varying calcium concentrations, as observed both in field studies and in laboratory rearing experiments with other pulmonate species (Hunter and Lull 1977; Nduku and Harrison 1980). *Physa skinneri* inhabiting softwater systems might maintain high levels of internal calcium at the expense of not allocating scarce calcium resources to shell growth, resulting in weaker shells. In other words, the shell may buffer the body from calcium loss, particularly in dilute environments. Although we examined populations from only four lakes, these lakes span most of the calcium concentration gradient available among Northern Highland lakes, the amount of variance explained was great, and there is a straightforward physiological explanation for the results.

Several factors each explained a relatively small but statistically significant amount of interpopulation variability in *H. anceps* shell strength. Calcium concentration was the most strongly correlated main effect, although it explained substantially less variability in *H. anceps* shell strength than in *P. skinneri* shell strength. This result is consistent with complementary findings that among-population differences in shell calcium content of *H. anceps* are not explained by environmental calcium (Hunter and Lull 1977). Predators may also have a small effect on shell strength. Shell strength was slightly greater in lakes with more predators, regardless of identity. Furthermore, the rate at which strength increased with size was faster in lakes with abundant pumpkinseed. If predators do influence *H. anceps* shell strength, it may be via induction rather than selection. Pulmonates (e.g., *H. anceps* and *P. skinneri*) secondarily reinvaded freshwaters from land, and they did so into smaller water bodies such as pond and ditch systems (McMahon 1983). This subclass of gastropods likely shares a shorter evolutionary history with large shell-damaging molluscivores, so selection for stronger shells would be less expected. The large amount of unexplained variation in the shell strength of this species may be accounted for by other factors. One hypothesis is that growth rates vary among lakes owing to differences in productivity (Hunter and Lull 1977). In faster-growing populations, tissue growth outstrips shell deposition, resulting in thinner shells (McMahon 1983). Genetic differences resulting from founder effects and nonadaptive drift also may underlie population differences in shell strength (sensu Russell-Hunter 1978).

Many physiological and life historical factors such as temperature tolerance, reproductive rate, metabolic efficiency, and bioenergetics vary among conspecific freshwater snail populations (Russell-Hunter 1978; McMahon 1983). Our findings add interpopulation differences in shell strength. Like the dimorphism seen in many species of marine intertidal gastropods, this variation may be correlated with environmental factors. For the snails studied here, the important factor and its strength of effect differ among species.

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References

Appleton, R.D., and Palmer, A.R. 1988. Water-borne stimuli released by predatory crabs and damaged prey induce more predator-resistant shells in a marine gastropod. Proc. Natl. Acad. Sci. U.S.A. **85**: 4387–4391.

Bates, D.M., and Watts, D.G. 1988. Nonlinear regression analysis and its applications. John Wiley & Sons, New York.

Baumgartner, J.V. 1995. Phenotypic, genetic, and environmental integration of morphology in a stream population of threespine stickleback, *Gasterosteus aculeatus*. Can. J. Fish. Aquat. Sci. **52**: 1307–1317.

Black, J.J., Andrews, L.M., and Threinen, C.W. 1963. Surface water resources of Vilas County. Wisconsin Department of Natural Resources, Madison, Wis.

Capelli, G.M., and Magnuson, J.J. 1983. Morphoedaphic and biogeographic analysis of crayfish distribution in northern Wisconsin. J. Crustacean Biol. **3**: 548–564.

Covich, A.P. 1977. How do crayfish respond to plants and mollusca as alternate food resources? Freshwater Crayfish, **3**: 165–179.

Covich, A.P., Crowl, T.A., Alexander, J.E., Jr., and Vaughn, C.C. 1994. Predator-avoidance responses in freshwater decapod–gastropod interactions mediated by chemical stimuli. J. North Am. Benthol. Soc. **13**: 283–290.

Crowl, T.A., and Covich, A.P. 1990. Predator-induced life-history shifts in a freshwater snail. Science (Washington, D.C.), **247**: 949–951.

Dodson, S. 1989*a*. Predator-induced reaction norms. BioScience, **39**: 447–452.

Dodson, S.I. 1989*b*. The ecological role of chemical stimuli for the zooplankton: predator-induced morphology in *Daphnia*. Oecologia, **78**: 361–367.

Draper, N.R., and Smith, H. 1981. Applied regression analysis. John Wiley & Sons, New York.

Havel, J.E. 1987. Predator-induced defenses: a review. *In* Predation: direct and indirect impacts on aquatic communities. *Edited by* W.C. Kerfoot and A. Sih. University Press of New England, Hanover, N.H.

Hunter, R.D. 1975. Variation in populations of *Lymnaea palustris* in upstate New York. Am. Midl. Nat. **94**: 401–420.

Hunter, R.D., and Lull, W.W. 1977. Physiologic and environmental factors influencing the calcium-to-tissue ratio in populations of three species of freshwater pulmonate snails. Oecologia, **29**: 205–218.

Johansson, F., and Samuelsson, L. 1994. Fish-induced variation in abdominal spine length of *Leucorrhinia dubia* (Odonata) larvae? Oecologia, **100**: 74–79.

Kenoyer, G.J., and Bowser, C.J. 1992. Groundwater chemical evolution in a sandy silicate aquifer in nórthern Wisconsin. 1. Patterns and rates of change. Water Res. **28**: 579–589.

Kershner, M.W., and Lodge, D.M. 1995. Effects of littoral habitat and fish predation on the distribution of an exotic crayfish, *Orconectes rusticus*. J. North Am. Benthol. Soc. **14**: 414–422.

Kitching, J.A., and Lockwood, J. 1974. Observations on shell form and its ecological significance in thaisid gastropods of the genus *Lepsiella* in New Zealand. Mar. Biol. **28**: 131–144.

Kratz, T.K., Webster, K.E., Bowser, C.J., Magnuson, J.J., and Benson, B.J. 1997. The influence of landscape position on lakes in northern Wisconsin. Freshwater Biol. **37**: 209–217.

Krueger, D.A., and Dodson, S.I. 1981. Embryological induction and predation ecology in *Daphnia pulex*. Limnol. Oceanogr. **26**: 219–223.

Lodge, D.M., Brown, K.M., Klosiewski, S.P., Stein, R.A., Covich, A.P., Leathers, B.K., and Bronmark, C. 1987. Distribution of freshwater snails: spatial scale and the relative importance of physicochemical and biotic factors. Am. Malacol. Bull. **5**: 73–84.

McMahon, R.F. 1983. Physiological ecology of freshwater pulmonates. *In* The Mollusca. Vol. 6: Ecology. *Edited by* W.D. Russell-Hunter. Academic Press, Inc., Orlando, Fla. pp. 359–430.

Mittelbach, G.G. 1984. Predation and resource partitioning in two sunfishes (Centrarchidae). Ecology, **65**: 499–513.

Mittelbach, G.G., Osenberg, C.W., and Wainwright, P.C. 1992. Variation in resource abundance affects diet and feeding morphology in the pumpkinseed sunfish (*Lepomis gibbosus*). Oecologia, **90**: 8–13.

Nduku, W.K., and Harrison, A.D. 1980. Cationic responses of organs and haemolymph of *Biomphalaria pfeifferi* (Krause), *Biomphalaria glabrata* (Say) and *Helisoma trivolvis* (Say) (Gastropoda: Planorbidae) to cationic alterations of the medium. Hydrobiologia, **68**: 119–138.

Nickerson, R.P. 1972. A survey of enzyme and shell variation in 16 populations of the stream limpet, *Ferressia rivularis* (Say). Ph.D. thesis, Syracuse University, Syracuse, N.Y.

Osenberg, C.W., and Mittelbach, G.G. 1989. Effects of body size on the predator–prey interaction between pumpkinseed sunfish and gastropods. Ecol. Monogr. **59**: 405–432.

Palmer, A.R. 1979. Fish predation and the evolution of gastropod shell sculpture: experimental and geographic evidence. Evolution, **33**: 697–713.

Rahel, F.J. 1984. Factors structuring fish assemblages along a bog lake successional gradient. Ecology, **65**: 1276–1289.

Russell-Hunter, W.D. 1978. Ecology of freshwater pulmonates. *In* Pulmonates. Vol. 2. Systematics, evolution and ecology. *Edited by* V. Fretter and J. Peake. Academic Press, London, U.K. pp. 335–383.

Russell-Hunter, W.D., Burky, A.J., and Hunter, R.D. 1981. Interpopulation variation in calcareous and proteinaceous shell components in the stream limpet, *Ferrissia rivularis*. Malacologia, **20**: 255–266.

Turner, A.M., Fetterolf, S.A., and Bernot, R.J. 1999. Predator identity and consumer behavior: differential effects of fish and crayfish on the habitat use of a freshwater snail. Oecologia, **118**: 242–247.

Vermeij, G.J., and Covich, A.P. 1978. Coevolution of freshwater gastropods and their predators. Am. Nat. **112**: 833–843.

Vermeij, G.J., Schindel, D.E., and Zipser, E. 1981. Predation through geological time: evidence from gastropod shell repair. Science (Washington, D.C.), **214**: 1024–1026.

Webster, K.E., Kratz, T.K., Bowser, C.J., Magnuson, J.J., and Rose, W.J. 1996. The influence of landscape position on lake chemical responses to drought in northern Wisconsin. Limnol. Oceanogr. **41**: 977–984.

Werner, E.E., Gilliam, J.F., Hall, D.J., and Mittelbach, G.G. 1983. An experimental test of the effects of predation risk on habitat use in fish. Ecology, **64**: 1540–1548.

Wilbur, K.M. 1976. Recent studies of invertebrate mineralization. *In* The mechanisms of mineralization in the invertebrates of and plants. *Edited by* N. Watabe and K.M. Wilbur. University of South Carolina Press, Columbia, S.C. pp. 79–108.

Wilkinson, L., Hill, M., and Yang, E. 1992. SYSTAT: statistics, version 5.2 edition. SYSTAT, Inc., Evanstan, Ill.

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DISCUSSION

Spatial autocorrelation and fish production in freshwaters: a comment on Randall et al. (1995)[1]

Caedmon H. Nash, John S. Richardson, and Scott G. Hinch

Spatial patterns are often present in results of ecological surveys (Legendre and Troussellier 1988; Legendre and Fortin 1989; Legendre 1993). When surveys span broad geographic areas, sites near one another often have similar levels of species abundance or biomass as a result of similar geologic, climatic, or biotic factors (Legendre and Fortin 1989). This positive autocorrelation at "small" spatial scales means that nearby sites should not be treated as independent replicates in classical statistical analyses (Legendre 1993). Under these conditions, statistical tests are too liberal and prone to type I errors. Despite knowing these pitfalls, many fisheries researchers continue to use classical statistical analyses and ignore the potential influence that autocorrelation could have on interpretations of broad geographic scale patterns (see Hinch et al. 1994) or temporal patterns (Pyper and Peterman 1998) of fish attributes.

In a recent issue of the *Canadian Journal of Fisheries and Aquatic Sciences*, Randall et al. (1995) used data from entire fish taxocenes for 31 lake and 62 river sites distributed throughout the world to suggest that relative to lakes, rivers had considerably greater fish production, density, biomass, and *P/B* and considerably lower individual fish mass. Although these results were compelling, we are concerned with the robustness of their conclusions. Because many of Randall et al.'s study sites were clustered within specific geographic locales (clusters of streams in Poland (14), England (nine), Malaysia (seven), and New Zealand (three) and clusters of lakes in Canada (12), Scandinavia (seven), and Russia (five)), we suspected that positive small-scale spatial autocorrelation exists in their data. If it does exist, then their statistical approach could be biased towards detecting differences between rivers and lakes (i.e., type I errors).

The purpose of this comment is to reevaluate the conclusions of Randall et al. in light of spatial autocorrelation. We accomplish this by examining their data for spatial patterns and then using two complementary analytical approaches for comparing fish attributes between rivers and lakes that are appropriate for spatially autocorrelated data. We have other concerns about their study, in particular, their sampling design is not globally well balanced; streams were clustered in northern Europe and the south Pacific, whereas no lakes were from these areas but were instead largely from Canada, Scandinavia, and Russia. There are no analytical approaches, including ones we suggest, that will remedy all biases in interpretation that may be caused by this sampling design. Further, some of the variables used by Randall et al. (e.g., production, biomass, weight, and *P/B*) are not independent of one another (an issue that has been discussed by Jackson et al. (1994)), yet were treated as independent variables for analytical and interpretative purposes. Moreover, field sampling in rivers and lakes requires different methods with different efficiencies that could bias their contrasts. However, we will not deal here with the sampling biases and independence issues listed above.

In our comment, we focus exclusively on the effects of spatial autocorrelation because there still seems to be a general misunderstanding or ignorance among aquatic ecologists about statistical approaches that are appropriate for broad geographic scale survey data. This is despite the publication over the past 10 years of papers that detail the potential pitfalls of spatial autocorrelation in ecological studies (e.g., Legendre and Troussellier 1988; Legendre and Fortin 1989; Legendre 1993; Hinch et al. 1994; Dunham and Rieman 1999). Spatial autocorrelation may be the result of important underlying environmental gradients and it is thus critical to consider such patterns explicitly. However, frequently, one is unaware of such gradients or the scale at which they influence patterns in nature, and autocorrelation analysis can at least expose the presence of such patterns. We are concerned here primarily with the biases that autocorrelation may impose on statistical inferences and not whether important gradients may be ignored as a consequence.

Testing for spatial autocorrelation

We used data from the appendices of Randall et al., and locations from the original references, to generate correlograms separately for lakes and rivers, using Moran's *I*

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[1]Randall, R.G., Kelso, J.R.M., and Minns, C.K. 1995. Fish production in freshwaters: are rivers more productive than lakes? Can. J. Fish. Aquat. Sci. **52**: 631–643.

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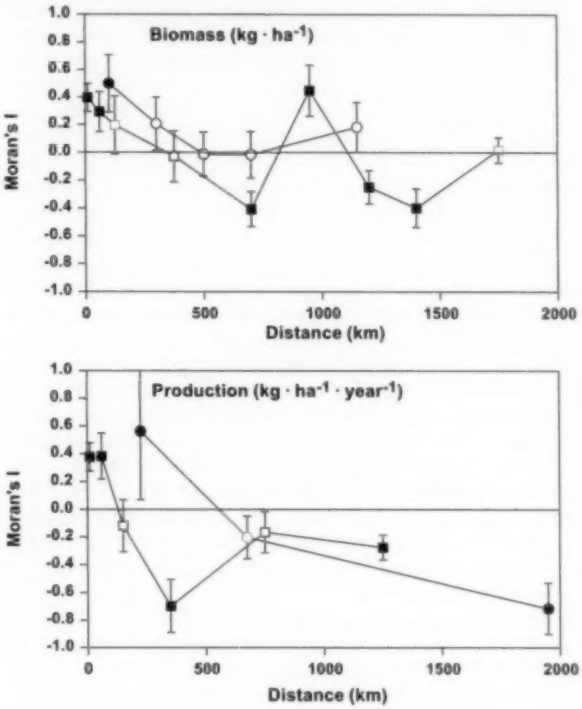
Fig. 1. Correlograms for fish biomass and production in rivers (squares) and lakes (circles) based on data from the literature compiled by Randall et al. Distance classes were chosen to ensure that at least 20 pairs of points were included for each. Positive values indicate positive autocorrelation and negative values indicate negative autocorrelation. Solid symbols are significant at $P = 0.05$ as determined by SAAP 4.3 (Wartenberg 1989); vertical bars indicate ±1 SD.



(Moran 1950). Correlograms were only generated for annual production and biomass, as these were the variables with the largest number of samples. Distances between pairs of sites (within lake or river categories) were calculated as straight-line distances using an algorithm assuming the earth to be a sphere with radius 6378.14 km (B. Klinkenberg, Geography, University of British Columbia, Vancouver, B.C., personal communication). Because the fish attribute measures were not normally distributed, all data were rank-transformed with the rank used as the variate for calculation of Moran's *I*. Distance classes were selected that contained no fewer than 20 point-pairs to obtain a maximum spatial resolution. Fewer point-pairs result in broad error estimates and thus reduce the power to detect differences. Fewer than 20 point-pairs may also be insufficient to assume normality, which is required for the use of Moran's *I* (Upton and Fingleton 1985). The selection of distance classes based on equal distances would not have allowed these assumptions to be met. Correlograms were generated with the Spatial Autocorrelation Analysis Program (SAAP 4.3) (Wartenberg 1989).

We found that biomass and annual production of fish, in both rivers and lakes, demonstrated positive autocorrelation at distances of less than or equal to 200 km and significant negative autocorrelation at distances greater than 1000 km (Fig. 1). At distances beyond 2000 km, the width of the distance class became too large to detect meaningful correlations. The positive autocorrelations for sites of close geographical proximity are likely a result of sampling within catchments, or within areas that exhibit similar watershed characteristics or climatic and geographic features (Hinch et al. 1994). Conversely, we found that sites separated by relatively large spatial scales tended to exhibit negative autocorrelation in biomass and production, but this was not consistent at all large distance classes. It is possible that this is simply an artifact of site selection, but we cannot rule out that at scales above 1000 km, differences in climate (e.g., temperate versus tropics) may account for some of the negative associations. Each correlogram had an overall significance of $P < 0.01$. The main point with respect to our comment is that there is strong, positive, small-scale spatial autocorrelation in these data that implies that many of the "replicates" from nearby locales are not truly independent.

Dealing with spatial autocorrelation

Spatial patterns in these data mean that "errors" are not necessarily independently distributed and as such, standard statistical approaches could generate erroneous results because the true probability of a type I error is unknown (Hurlbert 1984; Pyper and Peterman 1998). This problem of type I error rate is true even for the nonparametric analyses (i.e., Mann–Whitney *U* tests) used by Randall et al. (e.g., Stewart-Oaten 1995). Our first approach to achieve statistical inferences was with randomization *t* tests, in which significance was assessed by permuting the data repeatedly to compute a test statistic (Edgington 1987; Pyper and Peterman 1998). This approach does not require assumptions about the underlying error distribution, in contrast with the Mann–Whitney *U* tests used by Randall et al. Randomization tests do not resolve the spatial lack of independence, but do allow for analysis without depending on statistical tables generated under assumptions of no autocorrelation among data. We used the identical data set of Randall et al. (from their appendix) and compared lakes and rivers on estimates of annual production, species number (richness), fish density, individual fish mass, biomass, and *P*/*B*. Transformation of the data was not required, as normality is not an assumption of the randomization *t* test (Manly 1991). Tests were performed using a FORTRAN program modified from original code by Manly (1991). For each permutation, sites were randomly assigned to either the lake or river category while preserving the original proportions of the original data set. For each test, 50 000 permutations were performed to determine alpha probability levels. If the alpha level was close to 0.05, further permutations were carried out until values stabilized.

We found no significant difference ($P = 0.133$, Table 1) between lakes and rivers in fish biomass, in contrast with Randall et al. who reported a strong difference ($P = 0.005$). Like Randall et al., we found that production, density, and *P*/*B* were greater in rivers relative to lakes. However, the probability levels that we determined were much lower for production ($P = 0.050$) and density ($P = 0.010$) than their results (both $P < 0.001$), suggesting a much lower level of

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Table 1. Means, sample sizes, and ratio of means of fish attributes in the lakes and rivers examined by Randall et al. (original data) and in the lake and river clusters that we derived from their data.

	Sample size (original data)		Sample size (clusters)		Mean (original data)			Mean (clusters)			*P* value for Mann–Whitney tests, original data	*P* value for randomization tests	
Attribute	Lakes	Rivers	Lakes	Rivers	Lakes	Rivers	River to lake ratio	Lakes	Rivers	River to lake ratio		Original data	Clustered data
Production (kg·ha^{-1}·year^{-1})	22	55	15	15	81.8	273	3.34	91.9	403	4.39	<0.001	0.050	0.070
Species (no.)	20	47	11	13	6.3	9.4	1.49	6.4	7.4	1.16	0.254	0.111	0.671
Density (no.·ha^{-1})	19	42	11	9	5577	75 665	13.57	8178	62 859	7.69	<0.001	0.011	0.066
Individual mass (g)	19	42	11	9	28.9	4.1	0.14	25.1	5.2	0.21	<0.001	<0.001	<0.001
Biomass (kg·ha^{-1})	31	58	18	20	83.8	146	1.74	94.4	197	2.09	0.005	0.133	0.241
P/B	22	51	15	14	0.78	1.63	2.09	0.91	1.54	1.69	<0.001	<0.001	0.050

Note: The *P* values for Mann–Whitney *U* tests are from Randall et al.

confidence in the true differences between lakes and rivers. Like Randall et al., we also found that richness did not differ ($P = 0.111$) between lakes and rivers and that individual mass was much greater ($P < 0.001$) in lakes than in rivers.

Our second approach focused on creating a more balanced contrast between lakes and rivers based on grouping of sites that were geographically close to one another. To do this, we averaged fish attribute data, among rivers and lakes separately, from sites that clustered within 200 km of each other. This was the smallest spatial extent at which positive autocorrelation was detected with the correlograms (Fig. 1). The maximum distance within a lake cluster was only 26 km, and the maximum distance within a river cluster was 183 km. This averaging approach is more conservative than our randomization test. However, it is appealing because it likely reveals a more accurate estimate of the correct degrees of freedom (see cluster sample sizes in Table 1). If true differences exist between rivers and lakes, we would expect the results to be robust to how data are aggregated, whereas differences from the original results would suggest an artifact of the data structure by overrepresentation of particular geographic areas. Furthermore, averaging is an intuitive method. Randall et al. adopted this approach for one of their lake data points that was generated by averaging fish attribute data collected from 19 small, similarly sized Ontario lakes; their rationale for this action was that these lakes were considered as "replicates." Our method for aggregating sites was based on the scale at which positive autocorrelation was found rather than lumping sites based on political boundaries, such as all lakes in Canada, which would have included systems with disparate characteristics in the same group.

With our clustering approach, the number of lake and river clusters varied between nine and 20 depending on the variable (Table 1). This resulted in most of our variables having nearly identical sample sizes in lakes and rivers (Table 1). We employed randomization *t* tests to compare estimates of annual production, species richness, fish density, individual fish mass, biomass, and *P/B*. Tests were performed as stated above, except that for each permutation, cluster averages were randomly assigned to either the lake or river category.

We found no difference ($P = 0.241$, Table 1) between lake and river clusters in fish biomass, in contrast with Randall et al. who reported a strong difference between lakes and rivers ($P = 0.005$). Unlike Randall et al., who found that rivers had substantially higher production, density, and *P/B* relative to lakes (all $P < 0.001$), we found that river clusters had only marginally higher values of production ($P = 0.070$), density ($P = 0.066$), and *P/B* ($P = 0.050$) than lake clusters. Similar to Randall et al.'s results, we found that richness did not differ ($P = 0.671$) between lake and river clusters. Individual mass was the one fish attribute that both we and Randall et al. found to be much greater ($P < 0.001$) in lakes than in rivers; however, we found the magnitude of the differences between individual mass in lakes and rivers to be about 25% less than they found (Table 1).

Conclusions

Are rivers more productive for fish than lakes? After min-

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imizing the influence of spatial autocorrelation that was inherent in Randall et al.'s data, the answer is equivocal. In agreement with Randall et al., we found that lakes had substantially higher individual fish mass than did rivers, and we found that there was no difference between lakes and rivers in species richness with both our analytical approaches. In contrast with Randall et al., we found no evidence with either of our analytical approaches that fish biomass differed between lakes and rivers. In addition, we found much weaker differences (compared with those found by Randall et al.) between lakes and rivers in fish production and density with both our analytical approaches. Although statistically weaker, these latter differences are still intriguing in that river clusters had about four times greater production and eight times greater density than lake clusters (Table 1). It is possible that statistically significant differences between rivers and lakes could be detected for these latter variables if one were first able to account for environmental covariables that may be contributing to among-lake or among-river differences. Future studies that can collate data from similar numbers of rivers and lakes that coexist in the same region, as suggested by Randall et al., and that can select replicate sites that are truly independent, thus reducing the bias of the study, may help to confirm hypotheses about the relative productivity of fish in rivers and lakes. If sites cannot be selected to be independent, then appropriate statistical models (e.g., randomization approaches) will be needed. Until such a study is carried out, aquatic ecologists should exercise caution when generalizing about differences in fish production between rivers and lakes.

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References

Dunham, J.B., and Rieman, B.E. 1999. Metapopulation structure of bull trout: influences of physical, biotic, and geometrical landscape characteristics. Ecol. Appl. **9**: 642–655.

Edgington, E.S. 1987. Randomization tests. Marcel Dekker, Inc., New York.

Hinch, S.G., Somers, K.M., and Collins, N.C. 1994. Spatial autocorrelation and assessment of habitat–abundance relationships in littoral zone fish. Can. J. Fish. Aquat. Sci. **51**: 701–712.

Hurlbert, S.H. 1984. Pseudoreplication and the design of ecological field experiments. Ecol. Monogr. **54**: 187–211.

Jackson, D.A., Harvey, H.H., and Somers, K.M. 1994. Ratios in aquatic sciences: statistical shortcomings with mean depth and the morphoedaphic index. Can. J. Fish. Aquat. Sci. **51**: 1788–1795.

Legendre, P. 1993. Spatial autocorrelation: trouble or new paradigm? Ecology, **74**: 1659–1673.

Legendre, P., and Fortin, M.-J. 1989. Spatial pattern and ecological analysis. Vegetatio, **80**: 107–138.

Legendre, P., and Troussellier, M. 1988. Aquatic heterotrophic bacteria: modeling in the presence of spatial autocorrelation. Limnol. Oceanogr. **33**: 1055–1067.

Manly, B.F.J. 1991. Randomization and Monte Carlo methods in biology. Chapman and Hall, New York.

Moran, P.A.P. 1950. Notes on continuous stochastic phenomena. Biometrika, **37**: 17–23.

Pyper, B.J., and Peterman, R.M. 1998. Comparison of methods to account for autocorrelation in correlation analyses of fish data. Can. J. Fish. Aquat. Sci. **55**: 2127–2140.

Randall, R.G., Kelso, J.R.M., and Minns, C.K. 1995. Fish production in freshwaters: are rivers more productive than lakes? Can. J. Fish. Aquat. Sci. **52**: 631–643.

Stewart-Oaten, A. 1995. Rules and judgement in statistics: three examples. Ecology, **76**: 2001–2009.

Upton, G., and Fingleton, B. 1985. Spatial analysis by example. Vol.1. Point pattern and quantitative data. Wiley, New York.

Wartenberg, D. 1989. SAAP — Spatial Autocorrelation Analysis Program. Rutgers University, Piscataway, N.J.

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PERSPECTIVE

Managing the decline of Pacific salmon: metapopulation theory and artificial recolonization as ecological mitigation

Kyle A. Young

Abstract: Pacific salmon (*Oncorhynchus* spp.) in the Pacific Northwest of North America have suffered regional declines and local extinctions primarily because of freshwater habitat destruction and overexploitation by fisheries. Management efforts to reverse this trend have correctly focused on habitat restoration and enhancement and stricter regulation of fisheries. Metapopulation theory and the ecology of the genus suggest that the addition of management efforts that artificially increase the rate of colonization of presently unoccupied habitats may promote the recovery and persistence of Pacific salmon in an ecologically realistic way. Such programs are conceptually and operationally different from traditional stock transfer and enhancement programs, which aimed to maintain a harvestable surplus of salmon in the face of habitat destruction and overfishing. I argue that artificial recolonization programs should be viewed as ecological mitigation, aimed at hastening the return of natural demographic and evolutionary processes, and hope here to promote an open discussion of their merits and risks as such.

Résumé : Les saumons du Pacifique (*Oncorhynchus* spp.) de la côte nord-ouest de l'Amérique du Nord ont connu des déclins régionaux et des extinctions locales principalement à cause de la destruction d'habitats en eau douce et de la surexploitation. Pour renverser cette tendance, les gestionnaires ont intelligemment mis l'accent sur la restauration et la mise en valeur des habitats, ainsi que sur un raffermissement de la réglementation des pêches. La théorie des métapopulations et l'écologie du genre laissent penser que les efforts de gestion qui accroissent artificiellement le taux de colonisation des habitats actuellement vacants peuvent favoriser un rétablissement durable des populations de saumon du Pacifique d'une manière écologiquement réaliste. Sur les plans conceptuel et opérationnel, les programmes de ce type sont différents des programmes classiques de transfert et de mise en valeur des stocks, qui visent à maintenir un excédent récoltable de saumon en dépit de la destruction des habitats et de la surpêche. Je suis d'avis que les programmes artificiels de recolonisation devraient être vus comme un effort d'atténuation des dommages environnementaux visant à accélérer le retour des processus démographiques et évolutionnaires naturels, et je souhaite que le présent article suscite un débat ouvert sur les mérites et les risques de ce type de programmes.

[Traduit par la Rédaction]

Introduction

The regional declines and local extinctions of anadromous Pacific salmon (*Oncorhynchus* spp.) in the Pacific Northwest of North America have come mainly by way of a double-edged anthropogenic sword: destruction of freshwater habitat and overharvest by commercial fisheries (Nehlsen et al. 1991; Slaney et al. 1996). Recent improvements in land use regulations should temper the rate of destruction of freshwater habitat, and permanent and temporary restrictions on commercial and sport fisheries should reduce marine mortality rates. As land and fisheries management practices come into accordance with a grim ecological reality, we are left waiting (hoping) for salmon populations to recover. The recovery of salmon populations will involve two related ecological processes: (*i*) existing populations will grow in size as freshwater habitat improves and fishery-induced mortality rates decline and (*ii*) new populations will be reestablished in unoccupied habitats by colonists straying from their natal streams during spawning migrations. Management efforts aimed at expediting this recovery have focused almost exclusively on the first process, primarily by creating or improving freshwater habitat in streams with existing salmon populations. Here, I use a metapopulation perspective to discuss the rationale for management programs that incorporate the second process. Specifically, I suggest that artificial recolonizations from existing populations into presently unoccupied habitats may in some cases hasten the recovery, and promote the persistence, of salmon populations in an ecologically realistic way.

I proceed with two purposes. The first is simply to present

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the role of artificial recolonization in salmon management from a metapopulation perspective. The second is to promote an open discussion of the use of artificial propagation as an ecological restoration tool, which, despite established protocols (Hard et al. 1992), remains a contentious subject among fisheries biologists charged with managing and restoring Pacific salmon populations and the ecosystems on which they depend (Waples 1999). I stress at the outset that artificial recolonization should not be viewed as a "quick-fix" alternative to habitat conservation and reduced harvest rates, which should together remain the focus of salmon management, but as an ecologically justifiable component of broader restoration efforts.

A metapopulation perspective

A metapopulation is a group of populations inhabiting discrete patches of suitable habitat that are connected by the dispersal of individuals between habitat patches (Hanski 1991) such that "there is sufficient migration among populations to have a significant impact on either the demography or genetic structure of each component population" (Stacey et al. 1997). The population structure of anadromous salmon fits reasonably well the characteristics of a metapopulation (Quinn 1993): asynchronous dynamics in local populations (size fluctuations, extinction, and colonization events are to some degree uncorrelated), and dispersal between populations is common enough to recolonize unoccupied habitat patches, but not so common as to violate the first requirement (Hanski 1991). Due to habitat destruction and fragmentation, this is perhaps more true today than historically (Li et al. 1995), when populations were probably more often structured as panmictic units or patchy populations (Harrison 1991, see below). The simplest metapopulation model considers a large number of habitable patches, where the proportion of patches occupied (p) at any time (t) depends on the proportion of patches occupied at time $t - 1$ and the rates of population extinction and colonization (Levins 1969). The rate of change in p is given by

$$(1) \qquad \frac{dp}{dt} = mp(1 - p) - ep$$

where m and e are the colonization and extinction probabilities, respectively. At equilibrium, $dp/dt = 0$ and the proportion of patches occupied is $p^* = 1 - e/m$, which requires that $e/m < 1$ for metapopulation persistence. To account for habitat loss, consider that a fraction $1 - h$ of the patches is destroyed. The number of patches suitable for colonization changes from $1 - p$ to $h - p$, and the rate of change in p is then (May 1991)

$$(2) \qquad \frac{dp}{dt} = mp(h - p) - ep$$

and the equilibrium solution becomes $p^* = h - e/m$, requiring now that $e/m < h$ for metapopulation persistence. The classic Levins model ignores spatial structure and within-patch population dynamics and assumes that all populations are identical regarding extinction and colonization probabilities. In fact, few natural metapopulations meet absolutely all the assumptions of the Levins model (Harrison 1991). Recent models account for spatial structure as well as variability in patch size and in extinction and colonization probabilities (Hanski 1997). The Levins model remains attractive because of its generality and qualitative agreement with the results of more realistic models. It is useful in the present context because it clearly identifies some of the key ecological processes related to the decline and potential recovery of Pacific salmon.

For what types of salmon population structure is reality approximated by this simple metapopulation model? Historically, pristine freshwater habitat and small-scale aboriginal fisheries probably resulted in low extinction rates (e) and migration rates (m) between populations so high that $e/m \cong 0$, meaning that $p^* \cong 1$. While population sizes may have fluctuated by orders of magnitude through time, only rare environmental catastrophes resulted in years with total recruitment failure. This situation may remain today for species with large spawning aggregations in unfragmented habitats (e.g., pink (*O. gorbuscha*) and chum salmon (*O. keta*) in remote areas of British Columbia and Alaska). In such situations, population structure may be better described as loosely structured panmixia, the Levins model is inapplicable, and artificial recolonization has little ecological or practical merit. Indeed, such situations are of relatively little conservation concern in general. Other population groups may have mainland–island or source–sink structures (Harrison 1991), where large (although perhaps declining) populations in high-quality patches provide a continual source of immigrants to smaller populations that might otherwise go extinct (the "rescue effect"). The assumptions of the model are most appropriate for species/populations whose habitat has been fragmented and whose numbers have declined due to habitat degradation and overfishing (e.g., coho salmon (*O. kisutch*) in the northwestern United States; also see Li et al. 1995). In these situations, any nondeterministic population extinction will result in $e/m > 0$, making the values of e and m potentially important in determining metapopulation persistence. It is for these types of population structure, particularly the latter, that the implications of the Levins model and the rationale for artificial recolonization are most relevant. In general, it is important to appreciate that population structure varies between and within species, and the ecological propriety of artificial recolonization programs will vary accordingly.

The equilibrium solution of eq. 2 and the associated inequality required for metapopulation persistence, that $e/m < h$, relate directly to the causes of salmon declines and potential restoration actions. The above inequality is often not satisfied in disturbed ecosystems; thus, some salmon metapopulations may presently be in a nonequilibrium state of "living dead," committed to eventual extinction in degraded and fragmented lotic networks (Hanski et al. 1996). That is, although we may observe p values > 0, the values of h, e, and m may actually prohibit a positive p^* at equilibrium. Metapopulation persistence can be promoted by increasing h, the fraction of original patches that are habitable, and (or) decreasing e/m. Habitat restoration efforts aim to increase h, while fisheries restrictions attempt to decrease e. In reality, h is neither necessarily binary (destroyed or habitable) or independent of e. Many patches have been merely degraded, which reduces the carrying capacity of the patch

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and the mean growth rate of the population, thus increasing the chance of population extinction due to demographic and (or) environmental stochasticity (Harrison 1991; Lande 1993). In this sense, ecological reality diverges from the assumptions of the simple Levins model. We are left with m, the probability of empty patches being colonized by migrants from extant populations. I suggest that along with management efforts to increase h and decrease e, we should consider attempts to increase m by artificially recolonizing suitable but presently unoccupied habitat patches.

I assume that unoccupied but habitable patches exist; at least some populations have gone extinct due to demographic or environmental stochasticity, or environmental catastrophes, not because their habitat patches are absolutely and permanently uninhabitable (deterministic extinction). Many population extinctions may have been in some sense deterministic, with freshwater habitat destruction rendering patches functionally uninhabitable and part of the quantity $(1 - h)$ above. Depending on future land management practices, such patches may recover naturally or with the aid of habitat restoration programs. Anthropogenic forces and natural stochastic forces do not influence extinction probabilities independently. As habitat deterioration reduces a patch's carrying capacity and population size, extinction by stochastic factors becomes more likely (Lande 1993). For example, overfishing can be related to demographic stochasticity; overfishing increases the chance that in a given year, no individual will produce offspring surviving to adulthood, especially in small populations. This is simply a demographic stochastic interpretation of the long-recognized susceptibility of small (declining) populations to mixed-stock fisheries. Similarly, land use practices can affect the frequency and severity of environmental stochasticities (e.g., flood flows, summer drought, water temperature) and catastrophes (e.g., landslides, debris torrents). An anthropogenic increase in the probability of recruitment failure due to environmental stochasticity or catastrophe is not the conceptual equivalent to a patch being deterministically (i.e., permanently) uninhabitable. Rather, it is an anthropogenic increase in e, the probability of a population going extinct. If $e = 1$ for a given population, an anthropogenic increase in extinction probability becomes at least functionally equivalent to deterministic extinction, and the patch is uninhabitable. As the patch recovers, it will become habitable but unoccupied, and the time until occupancy will depend on the colonization rate from neighboring patches. I know of no empirical data on natural extinction probabilities for salmon populations; the time scale of relevant processes is simply beyond that of most scientific studies. The claim that land management practices have resulted in deterministic extinctions, as well as increased the probabilities of stochastic and catastrophic extinctions, is supported by a substantial literature on aquatic ecosystem management in the Pacific Northwest.

Without intervention, anadromous salmon will naturally recolonize suitable but unoccupied habitat patches via adults straying to nonnatal streams (patches) during spawning migrations. Obvious examples of this process are the present distribution of anadromous salmonids in British Columbia, a region covered by ice during the penultimate glaciation, and the establishment of Pacific salmon populations following their introduction into the Great Lakes. Colonizations of empty patches at the basin and watershed scale have also been documented (see references in Groot and Margolis 1991; Quinn 1993; Unwin and Quinn 1993). How frequently and how far do potential colonists stray from their natal streams? Unfortunately, we know surprisingly little about straying rates in natural salmon populations, and information from hatchery populations (of which there is much more) is not necessarily transferable (Quinn 1993). Shapovalov and Taft (1954) studied two adjacent ocean tributaries in northern California (U.S.A.) and found that coho salmon strayed more often than steelhead trout (*O. mykiss*), about 20 versus 2% of spawners, respectively. Alternatively, Labelle (1992) found almost no straying by wild coho salmon to nearby ocean tributaries on Vancouver Island (Canada) (about 1% for two populations over 4 years). Tallman and Healey (1994), studying wild chum salmon, documented a high straying rate (54%) from one population into a nearby (2 km) ocean tributary. Unwin and Quinn (1993) found that chinook salmon (*O. tshawytscha*) from a New Zealand hatchery were more likely to stray within their natal river than to others. When fish did stray to other basins, those rivers in close geographic proximity and with high discharge were more likely to receive strays. Anomalously high straying rates may occur when natal streams have high suspended sediment loads resulting from disturbance events (e.g., volcanism, Leider 1989). In summary, intra- and inter-specific straying rates vary widely, increase as the definition of "home" becomes more specific (e.g., from basin to stream to stream reach), depend on the characteristics of the target stream, and may be episodic due to natural and anthropogenic disturbance events (Quinn 1993).

Artificial recolonization

Artificial recolonization involves removing adults from extant populations, breeding them in hatcheries, and planting the juveniles into unoccupied but habitable patches. All the standard caveats concerning aquaculture, stock transfers, and salmon management apply (Billington and Hebert 1991; Hard et al. 1992). Hatchery stocks should not be used for producing juvenile fish used in artificial recolonization programs. Care should be taken to prevent the transfer of diseases between watersheds. Never should so many adults be removed for breeding and recolonization that the persistence of extant (source) populations is threatened. Recolonization programs should be consistent with broader conservation objectives aimed at maintaining the perceived spatial patterns of intraspecific phenotypic and genetic diversity. Where appropriate, evolutionarily significant units (ESUs) and associated institutional guidelines can provide a framework for recolonization programs, although individual programs should be developed on a species- and site-specific basis (Hard et al. 1992). The natural ecology of Pacific salmon along with theoretical and observational information on the demographic and genetic effects of extinction and colonization in structured populations can guide the development of artificial recolonization programs. I briefly discuss some of the issues warranting attention before stressing the fundamental difference between artificial recolonization programs and traditional stock transfer and hatchery supplementation programs.

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Data on straying rates from extant populations suggest that removing 5–20% of adults from extant populations would be consistent with the genus' natural ecology (without intensive monitoring, we will not know the percentage or destination of naturally straying fish). Since the number of (adult) colonists is the product of straying rate and the size of the source population, large source populations are attractive because the demographic and genetic effects of removing adults for artificial propagation would be minimized. If maximizing the success rate of artificial colonizations is the goal, the source population should be from a stream geographically near and ecologically similar to the target patch. Efforts should be made to choose source populations with measurable phenotypic and life history traits, such as adult size and migration timing, that are likely to succeed in the target patch (Hard et al. 1992). This, however, will not guarantee that other, more subtle phenotypic traits will not limit recolonization success. As what healthy populations remain are generally in patches of favorable habitat, initial recolonization efforts should focus on unoccupied patches having similar habitat characteristics (Griffith et al. 1989). Also, successful recolonizations will be more likely when a large number of colonists are used and artificial recolonizations are repeated over a series of years (Griffith et al. 1989). This second point is particularly important in semelparous salmon because of the necessity to establish multiple year-classes in a new population.

While using source populations from patches that are geographically near and ecologically similar to target patches should increase the success rate of recolonizations, introducing fish into a diversity of patch types may promote metapopulation persistence. Single populations can go extinct for four nondeterministic reasons: demographic, genetic, and environmental stochasticity and environmental catastrophes (Harrison 1991). It is important to appreciate that if a population is so small that its persistence is threatened by genetic stochasticity, demographic and environmental factors represent more immediate threats to population persistence (Lande 1988). Environmental homogeneity among patches results in correlated responses to environmental stochasticity (e.g., drought, flood), which in highly variable environments can decrease the persistence time of a metapopulation (Harrison and Quinn 1989). Empirical evidence suggests that within-patch environmental heterogeneity can similarly increase population persistence time (Kindvall 1996). Thus, while focusing recolonization efforts on "quality" patches may result in more immediate successes, inclusion of a diversity of patch qualities or types, which should respond differently to environmental variation, may promote metapopulation persistence (Hanski 1997). This fact has been appreciated by fisheries ecologists who have stressed the importance of habitat diversity and natural disturbance regimes in developing habitat conservation strategies (Reeves et al. 1995). Interpatch environmental heterogeneity may be particularly important in salmon metapopulations because the demographic dynamics of adjacent populations are necessarily correlated by experiencing similar climatic and marine conditions.

The number of adults removed from extant populations for propagation and colonization of empty patches will depend on the demography of the source population(s) and the perceived/desired genetic structure of the metapopulation. Removing a large number of adults from extant populations, and utilizing stratified mating designs, will increase the actual and genetic effective population size (N_e) of juvenile populations used for recolonization. Since neutral genetic variation is lost at rate of about $1/2N_e$ per generation, maximizing N_e of colonizing juveniles will reduce the influence of founder effects, inbreeding, and genetic drift in causing nonadaptive evolutionary change in new populations. Maximizing N_e of founding populations will necessarily be balanced by the need to maintain sufficient N_es of source populations. Several authors have suggested that N_e of a few hundred is sufficient to maintain neutral genetic variation over the long term (e.g., Lande and Barrowclough 1987), while $N_e = 50$ may be acceptable over shorter time scales (but see Nunney and Campbell 1993). Maintaining N_es $\cong$ 100 in extant populations will require leaving several hundred spawners per year (Frankham 1995), so removing enough adults from single source populations to insure N_e = 50 in colonizing juveniles may be unrealistic when source populations are small. Nevertheless, removing adults from throughout the spawning season, stratified random breeding designs, and minimizing variation in fitness by controlling precolonization juvenile mortality can increase N_e of founding populations (Hard et al. 1992). It will be impossible to completely eliminate artificial selection during breeding and rearing in hatchery environments; we can only hope that minimizing the time of hatchery residency will result in natural selection after introduction being the more influential evolutionary force.

The spatial, temporal, and demographic patterns of extinction, colonization, and migration affect the genetic structure of metapopulations (Slatkin 1987). If there is genetic differentiation among populations, extinction without recolonization will reduce the total genetic diversity of the metapopulation. The proportion of the total neutral genetic variation that is between populations is usually measured by F_{ST} or G_{ST}, the multiallele equivalent of F_{ST}. Altukhov and Salmenkova (1994) found an average $F(G)_{ST}$ of 0.07 (SD = 0.04) for North American salmon ($n = 8$), a value similar to estimates of G_{ST} for anadromous species in general (mean = 0.11, SD = 0.04, $n = 11$, Ward et al. 1994). Thus, local extinctions in Pacific salmon have probably resulted in a loss of total genetic diversity. The F_{ST} values for Pacific salmon estimated from molecular data are often higher than those predicted from assuming that observed straying rates represent genetic effective migration rates (based on the approximation $F_{ST} \cong 1/(4N_em + 1)$, where N_em is the number of genetic effective migrants entering a population) (but see Altukhov and Salmenkova 1994). This could occur if straying rates are higher than actual genetically effective migration rates and (or) if the alleles assayed are subject to dispersive selection.

The genetic effective number of individuals (k) used to colonize empty patches can influence F_{ST}. Generally, if $k < 2N_em$ (where N_em is the number of genetic effective migrants entering extant populations), then extinction and recolonization will diminish effective gene flow among populations and increase F_{ST} (Wade and McCauley 1988). This condition is based on the "migrant pool" model, where colonists are drawn randomly from all extant populations,

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and changes to $k < 2N_em/(1 - \phi)$ when colonists are drawn from select populations (where ϕ is the probability that two alleles come from the same source population, and $\phi = 0$ reduces to the above migrant pool condition) (Whitlock and McCauley 1990). Thus, removing adults from a single population ($\phi \rightarrow 1$, the "propagule pool" model) will always increase F_{ST} if $m > 0$, while colonizations originating from multiple populations may or may not promote interpopulation genetic differentiation, depending on the relative values of k and N_em. By manipulating the source(s) and genetic effective size of colonizing populations, artificial recolonization programs can potentially influence the distribution of genetic diversity in ways consistent with the measured or perceived genetic structure of different metapopulations.

The preceding discussion relates to neutral genetic variation, that which arises not from a response to natural selection but from the balance between migration, founder effects, drift, and mutation. A common assumption in salmon management is that different populations have adapted to variable environmental conditions and that local extinctions represent losses not only of neutral genetic diversity but of genetic combinations and phenotypic traits favored by spatially variable natural selection. While almost surely the case, the combination of relatively high straying rates and small effective population sizes may in some cases prevent local adaptation in salmon populations unless selection coefficients are sufficiently high (Adkison 1995). Unfortunately, information on adaptive genetic diversity, effective migration rates, and the strength selection in natural salmon populations is presently insufficient to test relevant theory. Until more is known about the relationship between genetic differentiation and local adaptation, a conservative approach is recommended. Recolonization programs should treat interpopulation genetic differentiation as representing at least the potential for local adaptation.

Discussion

In the end, we must weigh the demographic and genetic risks and benefits of artificial recolonization programs against those of inaction. A metapopulation perspective suggests that artificially increasing colonization rates to empty but habitable patches may in some cases hasten the recovery of Pacific salmon in an ecologically realistic way. The genetic risks from the responsible implementation of such programs may be less immediate than the benefits of reducing the risk of population and metapopulation extinction from demographic and environmental factors (Lande 1988; Hanski 1997). Inaction will leave salmon to recolonize empty but habitable patches naturally, meaning fewer populations and lower colonization rates in a necessarily less heterogeneous matrix of patches for a longer period of time. Salmon (meta)populations will remain relatively more susceptible to local extinction from genetic, demographic, and environmental factors. As presently uninhabitable patches recover naturally and (or) via restoration efforts, the ecological rationale for artificial recolonization should become more broadly justified.

"Translocation" is a well-established and often successful component in conservation management plans for other endangered species (Griffith et al. 1989), and artificial recolonization in a variety of forms is already used by salmon managers throughout the Pacific Northwest. Despite the cases of successful colonizations mentioned earlier, the litany of failed colonizations using both wild and hatchery populations gives reason for pause (see chapters in Groot and Margolis 1991). For "wild" translocations within their native range, a clear and not surprising pattern emerges for Pacific salmon. When source and target patches are geographically distant and ecologically dissimilar, failure appears almost guaranteed; when source and target patches are close geographically and similarly ecologically, successful colonizations are more frequent. The obvious lessons of these failures and successes should continue to guide any future artificial recolonization programs. Many of the fragmented and degraded lotic ecosystems in the Pacific Northwest of the United States contain large hatcheries that produce adults that stray to natural streams during migration. These strays have generally failed to establish naturally regenerating populations in what often appear to be "empty but habitable patches"; this situation is a classic example of a source–sink population structure. I believe that these failures argue not against the likelihood of successful colonization by wild populations but, along with historic stock transfer failures, for the importance of considering local adaptation in planning artificial colonization programs. Clearly, the goals of existing hatchery operations need to be considered (even changed) when deciding whether artificial recolonization programs are an appropriate management tool. These same hatcheries will in many cases provide the necessary infrastructure for the implementation of such programs.

At what spatial scale will artificial recolonization be an ecologically responsible and effective management tool? While implementation will require a case-specific approach, a conceptual hierarchy of population structure provides a useful framework (see Hard et al. 1992; Waples 1995). The essential question is at what spatial scale does a metapopulation perspective apply to salmon in anthropogenically degraded and fragmented environments? The ESU concept is based on historic levels of gene flow over evolutionary time scales and generally groups multiple basins together as "species" for consideration under the Endangered Species Act of the United States. Except for cases where single basins or stocks are designated as ESUs, artificial recolonization will be appropriate at spatial scales below ESUs (Hard et al. 1992). The traditional stock concept may represent a scale at which population demographics are influenced by migration and extinction dynamics in degraded and fragmented lotic networks. A (group of) watershed(s) may have one or more stocks (metapopulations), each comprising numerous patches (streams or stream segments), some of which may have extant populations, some of which may be empty but habitable, others of which may be uninhabitable. The spatial scale of implementation remains dependent on the definition of stock and population, which will vary within and between species and may be refined with field and (or) molecular estimates of population structure.

Two final points concerning artificial recolonization programs require mention. First, such programs should not proceed with the thoughtless zealotry that characterized

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traditional hatchery programs and resulted in the ill-conceived, and often unsuccessful, propagation and transplant of hatchery (and "wild") populations within and between basins. I offer a clear distinction between the two. Traditional aquaculture has been largely economic mitigation (supplementation) in an attempt to maintain commercial and sport fisheries in the face of habitat degradation and overharvest. Artificial recolonization programs should be implemented as ecological mitigation, with the singular goal of hastening the return of natural ecological and evolutionary processes. Second, artificial recolonization programs should proceed with an experimental spirit. The anadromous salmon system has much to offer the fields of evolutionary ecology, metapopulation dynamics, and landscape ecology and may help facilitate their eventual and necessary conceptual merger. For example, how do the number of colonists and environmental differences between source and target patches influence the establishment and growth of new populations? How does spatiotemporal variation in natural disturbance regimes (e.g., floods, droughts, landslides) interact with intra- and inter-patch environmental heterogeneity to determine population and metapopulation persistence (Harrison and Quinn 1989; Kindvall 1996)? The existing infrastructure and substantial technical and financial resources available for salmon research create a unique opportunity to answer these and other relevant questions. Hopefully, well-designed artificial recolonization programs can expedite the recovery of wild salmon populations while simultaneously addressing questions of more general ecological and evolutionary interest.

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References

Adkison, M.D. 1995. Population differentiation in Pacific salmon: local adaptation, genetic drift, or the environment? Can. J. Fish. Aquat. Sci. **52**: 2762–2777.

Altukhov, Y.P., and Salmenkova, E.A. 1994. Straying intensity and genetic differentiation in salmon populations. Aquacult. Fish. Manage. **25**: 99S–120S.

Billington, N., and Hebert, P.D.N. (*Special Editors*). 1991. Special issue on the Ecological and Genetic Implications of Fish Introductions (FIN). Can J. Fish. Aquat. Sci. **48**(Suppl. 1).

Frankham, R. 1995. Effective population size/adult population size ratios in wildlife: a review. Genet. Res. **66**: 95–107.

Griffith, B., Scott, J.M., Carpenter, J.W., and Reed, C. 1989. Translocation as a species conservation tool: status and strategy. Science (Washington, D.C.), **245**: 477–480.

Groot, C., and Margolis, L. 1991. Pacific salmon life histories. University of British Columbia Press, Vancouver, B.C.

Hanski, I. 1991. Single-species metapopulation dynamics: concepts, models and observations. Biol. J. Linn. Soc. **42**: 17–38.

Hanski, I.L. 1997. Metapopulation dynamics: from concepts and observations to predictive models. *In* Metapopulation biology: ecology, genetics, evolution. *Edited by* I.A. Hanski and M.E. Gilpin. Academic Press, New York. pp. 69–92.

Hanski, I., Moilanen, A., and Gyllenberg, M. 1996. Minimum viable metapopulation size. Am. Nat. **147**: 527–541.

Hard, J.J., Jones, R.P.J., Delarm, M.R., and Waples, R.S. 1992. Pacific salmon and artificial propagation under the Endangered Species Act. NOAA Tech. Memo. NMFS-NWFSC-2.

Harrison, S. 1991. Local extinction in a metapopulation context: an empirical evaluation. Biol. J. Linn. Soc. **42**: 73–88.

Harrison, S., and Quinn, J.F. 1989. Correlated environments and the persistence of metapopulations. Oikos, **56**: 293–298.

Kindvall, O. 1996. Habitat heterogeneity and survival in a bush cricket metapopulation. Ecology, **77**: 207–214.

Labelle, M. 1992. Straying patterns of coho salmon (*Oncorhynchus kisutch*) stocks from southeast Vancouver Island, British Columbia. Can. J. Fish. Aquat. Sci. **49**: 1843–1855.

Lande, R. 1988. Genetics and demography in biological conservation. Science (Washington, D.C.), **241**: 1455–1460.

Lande, R. 1993. Risks of population extinction from demographic and environmental stochasticity and random catastrophes. Am. Nat. **142**: 911–927.

Lande, R., and Barrowclough, G.F. 1987. Effective population size, genetic variation, and their use in population management. *In* Viable populations for conservation. *Edited by* M.E. Soule. Cambridge University Press, Cambridge, U.K. pp. 87–123.

Leider, S.A. 1989. Increased straying by adult steelhead trout, *Salmo gairdneri*, following the 1980 eruption of Mount St. Helens. Environ. Biol. Fishes, **24**: 219–229.

Levins, R. 1969. Some demographic and genetic consequences of environmental heterogeneity for biological control. Bull. Entomol. Soc. Am. **15**: 237–240.

Li, H.W., et al. 1995. Safe havens: refuges and evolutionarily significant units. *In* Evolution and the aquatic ecosystem: defining unique units in population conservation. *Edited by* J.L. Nielson. Am. Fish. Soc. Symp. **17**: 371–380.

May, R.M. 1991. The role of ecological theory in planning reintroduction of endangered species. Symp. Zool. Soc. Lond. **62**: 145–163.

Nehlsen, W., Williams, J.E., and Lichatowich, J.A. 1991. Pacific salmon at the crossroads: stocks at risk from California, Oregon, Idaho, and Washington. Fisheries (Bethesda), **16**(2): 4–21.

Nunney, L., and Campbell, K.A. 1993. Assessing minimum viable population size: demography meets population genetics. Trends Ecol. Evol. **8**: 234–239.

Quinn, T.P. 1993. A review of homing and straying of wild and hatchery-produced salmon. Fish. Res. **18**: 29–44.

Reeves, G.H., Benda, L.E., Burnett, K.M., Bisson, P.A., and Sedell, J.R. 1995. A disturbance-based ecosystem approach to maintaining and restoring freshwater habitats of evolutionary significant units of anadromous salmonids in the Pacific Northwest. *In* Evolution and the aquatic ecosystem: defining unique units in population conservation. *Edited by* J.L. Nielson. Am. Fish. Soc. Symp. **17**: 334–349.

Shapovalov, L., and Taft, A.C. 1954. The life histories of the steelhead rainbow trout (*Salmo gairdneri*) and silver salmon (*Oncorhynchus kisutch*) with special reference to Waddell Creek, California, and recommendations regarding their management. Calif. Dep. Fish Game Fish Bull. No. 98.

Slaney, T.L., Hyatt, K.D., Northcote, T.G., and Fielden, R.J. 1996. Status of anadromous salmon and trout in British Columbia and Yukon. Fisheries (Bethesda), **21**(10): 20–35.

Slatkin, M. 1987. Gene flow and the geographic structure of natural populations. Science (Washington, D.C.), **236**: 787–792.

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Stacey, P.B., Johnson, V.A., and Taper, M.L. 1997. Migration within metapopulations: the impact upon local population dynamics. *In* Metapopulation biology: ecology, genetics, evolution. *Edited by* I.A. Hanski and M.E. Gilpin. Academic Press, New York. pp. 267–291.

Tallman, R.F., and Healey, M.C. 1994. Homing, straying, and gene flow among seasonally separated populations of chum salmon (*Oncorhynchus keta*). Can. J. Fish. Aquat. Sci. **51**: 577–588.

Unwin, M.J., and Quinn, T.P. 1993. Homing and straying patterns of chinook salmon (*Oncorhynchus tshawytscha*) from a New Zealand hatchery: spatial distribution of strays and effects of release date. Can. J. Fish. Aquat. Sci. **50**: 1168–1175.

Wade, M.J., and McCauley, D.E. 1988. Extinction and recolonization: their effects on the genetic differentiation of local populations. Evolution, **42**: 995–1005.

Waples, R.S. 1995. Evolutionary significant units and the conservation of biological diversity under the Endangered Species Act. *In* Evolution and the aquatic ecosystem: defining unique units in population conservation. *Edited by* J.L. Nielson. Am. Fish. Soc. Symp. **17**: 8–27.

Waples, R.S. 1999. Dispelling some myths about hatcheries. Fisheries (Bethesda), **24**(2):12–21.

Ward, R.D., Woodwark, M., and Skibinski, D.O.F. 1994. A comparison of genetic diversity levels in marine, freshwater, and anadromous fishes. J. Fish Biol. **44**: 213–232.

Whitlock, M.C., and McCauley, D.E. 1990. Some population genetic consequences of colony formation and extinction: genetic correlations within founding groups. Evolution, **44**: 1717–1724.

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OBITUARY / NÉCROLOGIE

In Memoriam: Dr. Peter John Schwinghamer

Peter Schwinghamer passed away peacefully at home in Halifax on 22 May 1999 at the age of 52 after a long and courageous battle with a brain tumour. Born in Saskatchewan, Peter was a prairie boy who became one of the world's leading benthic ecologists.

Peter received his Bachelor of Science degree (with distinction) from the University of Calgary in 1968 with a speciality in limnology. Soon after, he moved to Halifax and worked for the Canadian Wildlife Service as assistant regional limnologist. There he conducted limnological field surveys in six national parks in Atlantic Canada (Kejimkujik, Gros Morne, Fundy, Cape Breton, Terra Nova, and L'Anse aux Meadows). During this period, he earned a Masters Degree in Biology at Dalhousie University. In 1978, now with a wife and young family, Peter left the Canadian Wildlife Service and enrolled as a full-time graduate student at Dalhousie to pursue a PhD in Biology.

Immediately upon completing his PhD in 1981, Peter joined the Marine Ecology Laboratory, Department of Fisheries and Oceans, at the Bedford Institute of Oceanography (Dartmouth, N.S.) as a postdoctoral fellow. The following year he was recruited into a research scientist position. In 1987, following the dissolution of the Marine Ecology Laboratory, Peter transferred to the Northwest Atlantic Fisheries Centre (St. John's, Nfld.) where he established a new multidisciplinary program in benthic ecology for the Department of Fisheries and Oceans. In 1991, Peter was diagnosed with a brain tumour, underwent surgery, and made a remarkable recovery to continue his scientific career despite very trying personal circumstances.

While in Newfoundland, he held positions of honorary professor, Newfoundland Institute for Cold Ocean Science, and adjunct professor, Biology Department, Memorial University of Newfoundland. He also served as a highly respected Canadian representative on the Benthos Ecology Working Group of the International Council for the Exploration of the Sea (ICES). In addition, Peter served as an Associate Editor of the Canadian Journal of Fisheries and Aquatic Sciences from 1989 to 1992 and as Deputy Editor from 1992 to 1997. In 1995, Peter returned to the Bedford Institute of Oceanography where he worked until he was forced to retire early because of his medical condition.

Peter's scientific contributions were many. Although best known for his research into the size structure of marine benthic communities, his interests covered a broad range of topics in environmental science. His early work in limnology focused on conducting seasonal surveys of physical, chemical, and biological processes in lakes and streams that were used by Parks Canada to develop natural resource inventories and resource management plans.

His PhD thesis was on the biomass size structure of benthic communities. This revolutionary work, which built upon previous Canadian research on the size structure of pelagic

Nécrologie : Dr Peter Schwinghamer

Le 22 mai 1999, à Halifax, Peter Schwinghamer est décédé paisiblement dans son lit à l'âge de 52 ans, après une longue et courageuse bataille contre une tumeur au cerveau. Né en Saskatchewan, Peter était un garçon des Prairies, qui allait devenir un écologiste benthique parmi les plus renommés au monde.

En 1968, Peter a obtenu un baccalauréat ès sciences, (avec distinction) orientation limnologie, de l'Université de Calgary. Peu de temps après, il a déménagé à Halifax et a commencé à travailler pour le Service canadien de la faune en tant que limnologiste régional adjoint. Durant cette période, il a effectué des relevés limnologiques sur le terrain dans six parcs nationaux du Canada atlantique (Kejimkujik, Gros Morne, Fundy, Cap-Breton, Terra Nova et Anse aux Meadows) et a réussi une maîtrise en biologie de l'Université Dalhousie. En 1978, alors marié et père de famille, Peter a quitté le Service canadien de la faune et s'est inscrit comme étudiant à temps plein à l'Université Dalhousie pour obtenir un doctorat en biologie.

En 1981, dès l'achèvement de son doctorat, Peter a obtenu une bourse de recherches post-doctorales au Laboratoire d'écologie marine du ministère des Pêches et des Océans, à l'Institut océanographique de Bedford (Dartmouth, N.-É.). L'année suivante, il devenait chercheur scientifique. En 1987, après la dissolution du Laboratoire d'écologie marine, Peter a été transféré au Centre des pêches de l'Atlantique nord-ouest (St. John's, T.-N.) où il a établi un nouveau programme multidisciplinaire d'écologie benthique pour le ministère des Pêches et des Océans. En 1991, Peter a appris qu'il était atteint d'une tumeur au cerveau; il a subi une opération et a connu un rétablissement remarquable, ce qui lui a permis de continuer ses recherches en dépit de l'épreuve personnelle qu'il affrontait.

Lorsqu'il était à Terre-Neuve, Peter a occupé le poste de professeur honoraire au Newfoundland Institute for Cold Ocean Science et celui de professeur auxiliaire à la faculté de biologie de l'Université Memorial. Spécialiste très respecté, il a aussi représenté le Canada au sein du Groupe de travail sur l'écologie du benthos du Conseil international pour l'exploration de la mer (CIEM). De plus, Peter a été corédacteur en chef pour le Journal canadien des sciences halieutiques et aquatiques de 1989 à 1992, et de rédacteur adjoint de 1992 à 1997. En 1995, Peter est retourné travailler à l'Institut océanographique de Bedford jusqu'à ce qu'il soit obligé de prendre sa retraite pour cause de maladie.

Les contributions de Peter à la science sont nombreuses. Même s'il est principalement reconnu pour sa recherche sur la structure par taille des communautés benthiques marines, il était intéressé par une grande variété d'autres sujets reliés aux sciences de l'environnement. Ses premiers travaux en limnologie portaient sur la production de relevés saisonniers des processus physiques, chimiques et biologiques des lacs et des cours d'eau, relevés qui ont été utilisés par Parcs

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communities, has become a classic in benthic ecology. He proposed that benthic communities should be viewed as systems and analysed as such. He also predicted that benthic communities should display orderly, stable patterns of biomass body size distribution. He proceeded to quantify the size distribution of all taxa and size classes in several benthic communities from the Bay of Fundy and demonstrated that his prediction was correct. Benthic biomass spectra display peaks and troughs, the positions of which are remarkably constant features regardless of the total biomass and taxonomic composition of the community. These results brought benthic community analysis into the mainstream of ecological science. Moreover, Peter's observations of the benthos led to re-examination of pelagic spectra, revealing that much variability that had previously been dismissed as noise or sampling error was now perceived as carrying information on the structure of the systems.

Empirical and theoretical studies of benthic biomass size spectra continued to be the focus of his work while at the Marine Ecology Laboratory. His postdoctoral studies concentrated on developing improved methods for extracting and processing microfauna and meiofauna from marine sediments for biomass size structure studies. He proceeded to collect and process benthic samples from various marine environments including intertidal mudflats, beaches, a range of continental shelf locations, and abyssal silt-clays. This was done while participating in several large multidisciplinary projects funded to address the environmental impacts of tidal power development in the Bay of Fundy, the environmental impacts of deep-sea storage of high-level radioactive wastes, and the production of the southwest Scotian Shelf haddock stock. Clear patterns of benthic community size structure emerged from this work, as well as relationships between benthic and pelagic biomass size structure. He proposed that there was a direct correlation between the observed size classes of the benthic biomass peaks and the three predominant life styles imposed by the physical constraints of the sediment. His interpretation of these spectra opened up invaluable debate and interest over physical forcing versus life-history characteristics of dominant taxa as the primary determinants of species size distributions. It also pointed the way toward the contemporary formulation of aquatic production theory. For example, his work on biomass size spectra led to estimates of community production and respiration using allometric relationships. He also made major contributions to understanding carbon flow in estuaries, especially the role played by saltmarsh detritus. In collaboration with Canadian, Dutch, and U.K. colleagues, Peter played a leading role in the development of the Cumberland Basin ecosystem model, a carbon flow simulation model for an estuary at the head of the Bay of Fundy.

Upon moving to the Northwest Atlantic Fisheries Centre, Peter worked for several years studying the ecological factors affecting the expanding distribution of the toxic dinoflagellate *Alexandrium fundyense* around the coast of Newfoundland. This work produced practical information on the influence of resuspended cysts on outbreaks of paralytic shellfish poisoning.

Starting in 1990, Peter applied his broad experience in benthic ecology to the complex issue of the effects of mobile fishing gear on benthic habitat and communities. He sought

Canada pour établir des inventaires des richesses naturelles et des plans de gestion des ressources.

Sa thèse de doctorat portait sur la structure par taille de la biomasse des communautés benthiques. Cette thèse révolutionnaire, qui était basée sur des recherches canadiennes antérieures concernant la structure par taille des communautés pélagiques, est devenue un classique de l'écologie benthique. Peter y a émis l'hypothèse que les communautés benthiques seraient des systèmes et devraient donc être étudiées en tant que telles. Il y prédisait aussi que les communautés benthiques présenteraient des modèles ordonnés et stables de distribution par taille de la biomasse. Il a donc quantifié la distribution par taille de tous les taxons et de toutes les classes de taille dans plusieurs communautés benthiques de la baie de Fundy et a démontré la justesse de son hypothèse. Les spectres de la biomasse benthique présentent des crêtes et des creux, dont la position est remarquablement constante et indépendante de la biomasse totale et de la composition taxonomique de la communauté. Ces résultats ont ouvert à l'analyse des communautés benthiques le vaste domaine des sciences écologiques. De plus, les observations de Peter sur le benthos ont amené un réexamen des spectres pélagiques, qui a révélé que de nombreuses variations, considérées auparavant comme du bruit ou des erreurs d'échantillonnage, seraient porteuses d'informations sur la structure des systèmes.

Les études empiriques et théoriques sur le spectre des tailles de la biomasse benthique ont continué d'être le sujet des recherches de Peter au Laboratoire d'écologie marine. Ses études postdoctorales étaient axées sur l'amélioration des méthodes d'extraction et de traitement de la microfaune et de la méiofaune provenant des sédiments marins pour les études sur la structure par taille de la biomasse. Il a récolté et traité des échantillons benthiques de différents environnements marins, comme les vasières intertidales, les plages, une variété d'endroits sur le plateau continental, mais aussi les boues argileuses abyssales. Peter Schwinghamer a mené ces recherches tout en s'impliquant dans plusieurs gros projets multidisciplinaires, visant instamment les impacts environnementaux du développement de l'énergie marémotrice dans la baie de Fundy et ceux du stockage en eau profonde de déchets à radioactivité élevée, mais aussi la production du stock d'aiglefin du plateau néo-écossais. Des modèles clairs de la structure par taille de la communauté benthique sont ressortis de cette recherche, ainsi que des relations entre la structure par taille de la biomasse benthique et de la biomasse pélagique. Peter a posé qu'il existait une corrélation directe entre les classes de taille représentées par les crêtes de la biomasse benthique et les trois styles de vie prédominants imposés par les contraintes physiques des sédiments. Son interprétation de ces spectres a lancé un débat d'une valeur inestimable et suscité un intérêt sans pareil autour du rôle respectif des forces physiques et des caractéristiques du stade biologique des taxons dominants dans la distribution par taille des espèces. Elle a aussi préparé le terrain à la formulation contemporaine de la théorie sur la production aquatique. Par exemple, son travail sur le spectre des tailles de la biomasse a mené à des estimations de la production et de la respiration des communautés utilisant les relations allométriques. Peter a aussi contribué largement à la compréhension du flux de carbone dans les estuaires, en particulier

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to apply contemporary technological advances to the demanding task of providing adequate information about the structure of benthic communities. Working with colleagues at the Bedford Institute of Oceanography and in industry, he helped develop a suite of new and improved instruments for sensing and sampling benthic habitat and communities on offshore fishing banks. A major impediment to the study of benthic habitat structure has been the difficulty of studying undisturbed sediment structures and infaunal organisms. He collaborated with Guigné International Ltd. in Newfoundland in the development of DRUMS (Dynamically Responding Underwater Matrix Sonar), which utilized new technology in broad-frequency spectrum, narrow-beam acoustics to provide high-resolution measurements of undisturbed sediment habitat structure.

All of this new technology was applied to a unique 3-year experiment on the effects of otter trawling on a sandy bottom habitat, conducted at a carefully selected site on the Grand Banks. Peter played a leading role in the design and execution of this experiment, as well as in the analysis and interpretation of the data. His most significant contribution to this experiment was applying high-resolution acoustics and chaos theory to understand the effects of trawling on sediment habitat structure. The tools he helped develop during this project have had a major effect in redirecting current approaches to analyzing marine benthic communities. It was in the midst of these investigations that the tumour stopped him.

As a scientist, Peter possessed the invaluable qualities of being able to approach problems with an open mind and not hesitate to take a unique approach in their solution. This was often in the face of considerable opposition within a discipline that is often bound by traditional approaches. He was a very independent thinker who constantly and effectively challenged the status quo, was a continuous source of new ideas, and had innovative ways of addressing scientific questions. He was a stimulating person to be around. His intellect was acute and he could be tough and relentless in debate, but his tender side was manifest in all actions.

Peter was also a very compassionate individual who cared very much for other people, especially those who through no fault of their own found themselves in disadvantaged positions. His thoughtful intelligence was alloyed with compassion and love for his fellow humans. A devout Catholic, he was active in aiding refugees and establishing them in Canada. His hobbies included playing classical guitar, astronomy, camping, canoeing, and caving. However, of all his wonderful qualities, the most outstanding was his courage, especially in facing his long illness. He had an inner strength that few others possess and, with the devoted support of his family, kept a positive outlook up to the very end of his life. Peter was a man we all could wish to emulate. Kindness, compassion, and surpassing intellect are rare gifts in conjunction but they were all manifest in Peter.

Throughout his all-too-brief scientific career, Peter developed an excellent international reputation as an innovative benthic ecologist. One can only imagine what further contributions he could have made in the field of benthic ecology if his career had not been ended prematurely. One measure of a scientist's success is his influence on others. Peter excelled in this and freely communicated his enthusiasm and advice

l'importance des débris des marais salés. En collaboration avec des collègues du Canada, de la Hollande et du Royaume-Uni, Peter a joué un rôle prépondérant dans l'élaboration du modèle de l'écosystème du bassin de Cumberland, une simulation du flux de carbone pour un estuaire situé au fond de la baie de Fundy.

Au Centre des pêches de l'Atlantique nord-ouest, Peter a étudié pendant plusieurs années les facteurs écologiques qui influençaient l'expansion de la distribution du dinoflagellé toxique *Alexandrium fundyense* sur les côtes de Terre-Neuve. Cette recherche a fourni des renseignements pratiques à propos de l'influence de la remise en suspension des kystes sur les épidémies d'intoxication par la phycotoxine paralysante.

À partir de 1990, Peter a prêté sa vaste expérience en écologie benthique au dossier complexe des effets des engins mobiles sur les habitats et les communautés benthiques. Il a voulu utiliser les progrès techniques contemporains pour faciliter la collecte de renseignements adéquats sur la structure des communautés benthiques. En association avec des collègues de l'Institut océanographique de Bedford et de l'industrie, Peter a collaboré à la conception d'une gamme d'instruments nouveaux ou améliorés de détection et d'échantillonnage des habitats et des communautés benthiques sur les bancs de pêche hauturière. Un obstacle majeur pour la recherche sur la structure de l'habitat benthique a été la difficulté d'étudier les structures de sédiments et les organismes benthiques de milieux non perturbés. Peter a collaboré avec Guigné International Limited, à Terre-Neuve, par la conception du DRUMS (Dynamically Responding Underwater Matrix Sonar), une nouvelle technologie qui utilise l'acoustique à faisceau étroit et à large spectre de fréquence pour faire des mesures à haute résolution de la structure de l'habitat sédimentaire non perturbé.

Toute cette nouvelle technologie a été appliquée lors d'une remarquable expérience de trois ans concernant les effets du chalutage sur un habitat de fond sablonneux, réalisée à un endroit précis du Grand Banc de Terre-Neuve. Peter a joué un rôle prépondérant dans la conception et la réalisation de cette expérience, ainsi que dans l'analyse et l'interprétation des données. Sa contribution la plus importante a été l'application de l'acoustique de haute résolution et de la théorie du chaos à la compréhension des effets du chalutage sur la structure de l'habitat sédimentaire. Les outils qu'il a aidé à développer durant le projet ont eu un impact notable sur les approches actuelles de l'analyse des communautés benthiques marines. La tumeur est venue mettre prématurément un terme à ces recherches.

Peter possédait les qualités essentielles d'un scientifique; il savait s'attaquer aux problèmes avec un esprit ouvert et emprunter sans hésitation une nouvelle approche pour les résoudre. Il a souvent dû affronter une forte opposition dans un domaine entravé par son traditionnalisme. Il avait ses propres idées et remettait en question, constamment et avec efficacité, le statu quo; il était une source intarissable de nouvelles idées et trouvait des solutions inédites aux problèmes scientifiques. Sa compagnie était stimulante, il possédait un esprit vif et, s'il pouvait être coriace et inflexible lors d'un débat, son côté sensible savait toujours se manifester.

Peter était une personne très compatissante qui était à l'écoute des autres, particulièrement ceux qui, par les hasards de la vie, se retrouvaient défavorisés. Sa intelligence

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in a quiet, unassuming manner. Humour was always just below the surface, including a self-directed humour as to the perceived role of "mud and bug" people in the grand scheme of things. Peter had a positive influence on many colleagues and developing scientists around the world, all of whom will miss him but continue to gain inspiration through the legacy of his work and his strength of character.

We join his wife, Sandra, sons Timothy and Steven, and the rest of his loving family and friends in mourning the loss of Peter Schwinghamer. We are all the richer for having known him.

Donald C. Gordon, Jr. (DFO, Bedford Institute of Oceanography)
Kent D. Gilkinson (DFO, Northwest Atlantic Fisheries Centre)
Stephen J. Kerr (Dalhousie University)

profonde a été alliée avec une grande capacité de compassion et d'amour pour les autres humains. Catholique fervent, Peter était actif dans l'aide aux réfugiés et leur accueil au Canada. Dans ses loisirs, il pratiquait la guitare classique, l'astronomie, le camping, le canotage et la spéléologie. De toutes ses qualités merveilleuses, la plus remarquable était son courage, particulièrement face à la maladie. Il était animé d'une force intérieure que peu possèdent, et avec le support dévoué de sa famille, il a gardé son optimisme jusqu'aux tous derniers jours de sa vie. Peter était un modèle à suivre pour tous. Gentillesse, compassion et intelligence supérieure sont des qualités rarement réunies chez un même individu, mais Peter les possédait toutes.

Pendant sa trop brève carrière scientifique, Peter s'est forgé une excellente réputation internationale en tant qu'écologiste benthique doué d'une grande capacité d'innovation. Nous ne pouvons qu'imaginer ce qu'il aurait encore pu apporter au domaine de l'écologie benthique, s'il n'avait pas connu une fin prématurée. Si on mesure le succès d'un scientifique à son influence sur les autres, on peut affirmer que la carrière de Peter est une réussite totale; il partageait facilement son enthousiasme et donnait des conseils d'une manière discrète et modeste. L'humour pointait toujours à la surface, et il savait rire de lui-même et de la place, dans le vaste territoire des sciences, de son travail de spécialiste de « la vase et des vers ». Partout dans le monde, Peter a influencé positivement de nombreux collègues et chercheurs en herbe qui le regretteront, mais qui continueront à être inspirés par ses recherches et sa force de caractère.

Nous pleurons tous, avec sa femme, Sandy, ses fils, Tomothy et Steven, et le reste de sa famille et ses amis, la perte de Peter Schwinghamer. Nous sommes plus riches pour l'avoir connu.

Donald C. Gordon Jr. (MPO, Institut océanographique de Bedford)
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